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Introduction to Bioinformatics Course

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Acknowledgements

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Introduction

This course is designed to impress upon you that computers and the Internet can not only make your work as a biologist easier and more productive but also enable you to answer questions that would be impossible without computational help. Thus there are some computational analyses that you could conceivably do on the back of an envelope or with a pocket calculator and there are others so computationally demanding that you would not attempt them without electronic help. An example of the first would be to scan the following DNA sequence for ecoRI restriction endonuclease sites (GAATTC):

>Adhr *D.melanogaster*
ATGTTCGATTTGACGGGCAAGCATGTCTGCTATGTGGCGGATTGCGGAGGGAGACCAGC
AAGGTTCTCATGACCAAGAATATAGCGAAACTGGCCATTCGGAAAATCCCCAGGCCATC
GCTCAGTTGCAGTCGATAAAGCCGAGTACTTCTGGACCTACGACGTGACCATGGCAA**GA**
ATTCATATGAAGAAGTACTGATGGTCCAAATGGACTACATCGATGTCCTGATCAATGGT
GCTACGCTGATAACATTGATGCCACCATCAATACAAATCTAACGGGAATGATGAACACG
TGTTACCCTATATGGACAGAAAAATAGGAGGAATTCGTGGGCTTATTGTTCGGTCATTG
GATTGGACCCTTCGCCGGTTTTCTGCGCATATAGTGCAGTGTAATTGGATTTACCAGAA
GTCTAGCGGACCCTCTTTACTATTCCCAGCTGTGATGGCGGTTTGTTGTGGTCCTACAA
GGGTCTTTGTGGACCGGGGTTTTTAGAATACGGACAATCCTTTGCCGATCGCCTGCGGC
GAGCGCCCCATCGGTTTGTGGTCAGAATATTGTCAATGCCATCGAGAGATCGGAGAATG
GATTGCGGATAAGGGTGGACTCGAGTTGGTCAAATTGCATTGGTACTCGACCAGTTCGT
GCACTATATGCAGAGCAATGATGAAGAGGATCAAGAT

(This sequence is written in Fasta format - see below for sequence formats.)

A computer could do it quicker, but it is still trivial to do it by eye. Especially as one of the sites has been picked out in bold. Can you find the other(s)? Sequence analyses impossible without a computer include, but are not limited to, most operations that involve the sequence databases. The DNA databases (Genbank EMBL DDBJ) are curated by three different groups in Bethesda, MD, Hinxton, UK and Mishima, JP but, because they exchange information on a daily basis, should be effectively the same in content. The DNA databases are doubling in size about every year; they currently (Oct 2008) comprise:

> 90 million sequences

and

99,116,431,942 base pairs

So finding all of the ecoRI sites in GenBank or even the whole of a printed copy of the human genome (3,200,000,000 bp) would take more than a few minutes.

This course will introduce you to some of the more commonly used bioinformatics tools; tell you how to use them and, more importantly, how to use them "correctly" or at least more effectively. Most of the analysis will be carried out on the World Wide Web

(WWW). This is partly because it is available to all comers without requiring direct access to the necessary computers, which serve as database and software repositories. But it is also partly because a well-designed Web site can be particularly user-friendly and intuitive in its operations.

There are likely to be network related problems trying to make 25 simultaneous connections over the Internet to the same site. Try doing the course exercises late in the evening, early in the morning (best for speed!) or at weekends.

This module in bioinformatics is designed to give you a flavour of what analytical and informative tools are available on the World Wide Web.

Software used in the course are many and varied. We have tried to put links to them all on the course website:

http://hpc.ilri.cgiar.org/ILRI2009/

A few overall points for the course:

- Take the opportunity to compare and contrast different methods of doing a particular analysis.
- By all means take the defaults but be aware that changing them will almost certainly get more or better information.
- The Web is free, and you get what you pay for, so use the Web with care & caution.
- As with lab work it takes time to get the protocol working. Once you have one that works for you, write it down, bookmark and remember it. But note, the Web changes rapidly and you cannot afford to use outmoded technology for long.
- Where applicable we will also introduce you to the same tool implemented in the EMBOSS package. EMBOSS is a free Open Source software analysis package specially developed for the needs of the molecular biology user community. EMBOSS integrates a range of currently available packages and tools for sequence analysis into a seamless whole. The EMBOSS package will be described in detail in a separate course module.

Introduction to Bioinformatics

Bioinformatics has been described as the storage, retrieval and analysis of biological sequence information. In this short course we will be taking a broader definition: how computers can maximise the biological information available to you. This will touch on determining the 3-D structure of bio-molecules and trying to relate this to their function as well as accessing the relevant literature. I hope that, by the end of the course, everyone will be adopting a more explicitly evolutionary understanding of ‘their’ molecule. The formal course practicals can be carried out entirely on the World Wide Web using Netscape or the other Web-browser. Nevertheless, we recommend using locally installed (FREE) software for the phylogenetic trees part of the course.

You should note that several important types of bioinformatic analysis are not freely accessible on the Web, but are available on various password controlled computers. In particular, types of analysis that require large amounts of computational power/time are best carried out off the web. Analyses of many genes are also often better done in an environment where a computer program does the pointing and clicking for you. For the record, the EMBOSS package is a suite of programs which carry out almost all the analyses that a molecular biologist might want to do with/on DNA or protein sequences (secondary structure prediction, two sequence alignment, conceptual translation of DNA, restriction site analysis, primer design, as well as homology searching, multiple sequence alignment etc.). For phylogenetic inference and tree drawing, the PHYLIP package (versions available for PCs, Macs and Unix) will answer most needs. Both of these software packages and a variety of other sequence analysis packages are available for download from the Internet.

The web, by contrast, is a total mess: the same program is implemented with different defaults at different sites; it is often not clear what those defaults, options and parameters are; the results are not easily transferred to a different program. So it is free, but there is a cost! You are advised to validate any analysis against the results yielded by other sites.

For a good introduction to Bioinformatics, read the first chapter of Developing Bioinformatics Computer Skills, Cynthia Gibas & Per Jambeck, available online at http://oreilly.com/catalog/bioskills/chapter/ch01.html

Databases

Databases are of course the core resource for bioinformatics. There is plenty of software for analysing one or a few sequences, but many of the computationally interesting and biologically informative programs access databases of information. Frequently used classes are the biological sequence databases. These include:

- EMBL (European Mol Biol Lab)
- GenBank
- DDBJ (DNA DB of Japan)

These three DNA databases exchange their data on a daily basis and so should be identical as to content. They are, however, rather different in format:
Each of the database cited above consists of a (very large number) of entries, each consisting of a single sequence preceded by a quantity of 'annotation' that puts the sequence in its biological, functional and historical context. Without the annotation, GenBank would be a meaningless string of 32 billion As Ts Cs and Gs. Compare and contrast the two extracts from a) EMBL and b) Genbank (DDBJ has the same look-and-feel as Genbank):

a) EMBL

```
ID ECRECA standard; DNA; PRO; 1391 BP.
AC V00328; J01672;
DT 09-JUN-1982 (Rel. 01, Created)
DT 12-SEP-1993 (Rel. 36, Last updated, Version 4)
DE E. coli recA gene.
KW .
OS Escherichia coli
OC Bacteria; Proteobacteria; gamma subdiv; Enterobacteriaceae;
OC Escherichia.
RN [1]
RP 1-1374
RX MEDLINE; 80234673.
RA Sancar A., Stachelek C., Konigsberg W., Rupp W.D.;
RT "Sequences of the recA gene and protein";
RL Proc. Natl. Acad. Sci. U.S.A. 77:2611-2615(1980).
```

b) GenBank

```
LOCUS ECRECA 1391 bp DNA BCT 12-SEP-1993
DEFINITION E. coli recA gene.
ACCESSION V00328 J01672
KEYWORDS .
SOURCE Escherichia coli.
ORGANISM Escherichia coli
Eubacteria; Proteobacteria; gamma subdiv; Enterobacteriaceae;
Escherichia.
REFERENCE 1 (bases 1 to 1374)
AUTHORS Sancar,A., Stachelek,C., Konigsberg,W. and Rupp,W.D.
TITLE Sequences of the recA gene and protein
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 77 (5), 2611-2615 (1980)
```

You can see that these two are obviously talking about the same sequence from *E.coli*, but the information is encoded in a rather different way. This makes no difference to us reading the text, but causes problems when writing a program to interrogate a database.

Each database entry has a name, called ID or LOCUS, which tries to be mnemonic and marginally informative. More importantly each has an accession number which is arbitrary but which remains attached to the sequence for the rest of time. The organism might become reclassified, the gene may get renamed and the ID is thus subject to change, but by noting the accession number you should always be able to identify and retrieve the sequence. Note also that the original publication is cited. Usually there will be other papers documenting functional analysis, mutations, allelic variations, 3-D structure and so on.

Further down in the entry is annotation about the sequence itself, so that the sequence is parsed into meaningful bits called a features table:

a) EMBL

```
FT source 1. .1391
FT /organism="Escherichia coli"
FT /db_xref="taxon:562"
FT mRNA 191. .>1391
FT /note="messenger RNA"
FT RBS 229. .233
FT /note="ribosomal binding site"
FT CDS 239. .1300
FT /db_xref="SWISS-PROT:P03017"
FT /transl_table=11
FT /gene="recA"
FT /product="recA gene product"
FT /protein_id="CAA23618.1"
FT
FT mutation 353. .353
FT /note="g to a in recA441 (E to K)"
FT mutation 720. .720
FT /note="g to a in recA1 (G to D)"
```

b) GenBank

```
FEATURES Location/Qualifiers
source 1..1391
/organism="Escherichia coli"
/db_xref="taxon:562"
mRNA 191..>1391
/note="messenger RNA"
RBS 229..233
/note="ribosomal binding site"
gene 239..1300
/gene="recA"
CDS 239..1300
/gene="recA"
/codon_start=1
/transl_table=11
/product="recA gene product"
/db_xref="SWISS-PROT:P03017"
mutation 353
/gene="recA"
/note="g to a in recA441 (E to K)"
mutation 720
/gene="recA"
/note="g to a in recA1 (G to D)"
```

Again you can see that the information exchange between Genbank and EMBL includes all significant portions of the annotation. Such useful signals and data as the open reading frame (CDS for CoDing Sequence), the ribosome binding site, intron boundaries, signal peptides, variants/mutations may be recorded.

Protein databases:

- SwissProt
- PIR (Protein Information Resource)
- GenPept

a) Swissprot

```
ID RECA_ECOLI STANDARD; PRT; 352 AA.
AC P03017; P26347; P78213;
DT 21-JUL-1986 (REL. 01, CREATED)
DT 21-JUL-1986 (REL. 01, LAST SEQUENCE UPDATE)
DT 15-DEC-1998 (REL. 37, LAST ANNOTATION UPDATE)
DE RECA PROTEIN.
GN RECA OR LEXB OR UMUB OR RECH OR RNMB OR TIF OR ZAB.
OS ESCHERICHIA COLI, AND SHIGELLA FLEXNERI.
OC BACTERIA; PROTEOBACTERIA; GAMMA SUBDIVISION; ENTEROBACTERIACEAE;
OC ESCHERICHIA.
...
...
CC -!- FUNCTION: RECA PROTEIN CAN CATALYZE THE HYDROLYSIS OF ATP IN THE
CC PRESENCE OF SINGLE-STRANDED DNA, THE ATP-DEPENDENT UPTAKE OF
CC SINGLE-STRANDED DNA BY DUPLEX DNA, AND THE ATP-DEPENDENT
CC HYBRIDIZATION OF HOMOLOGOUS SINGLE-STRANDED DNAS. IT INTERACTS
CC WITH LEXA CAUSING ITS ACTIVATION AND LEADING TO ITS AUTOCATALYTIC
CC CLEAVAGE.
CC -!- INDUCTION: IN RESPONSE TO LOW TEMPERATURE. SENSITIVE TO
CC TEMPERATURE THROUGH CHANGES IN THE LINKING NUMBER OF THE DNA.
CC -!- DATABASE: NAME=E.coli recA Web page;
CC WWW="http://monera.ncl.ac.uk:80/protein/final/reca.htm".
KW DNA DAMAGE; DNA RECOMBINATION; SOS RESPONSE; ATP-BINDING; DNA-
BINDING;
KW 3D-STRUCTURE.
FT INIT_MET 0 0
FT NP_BIND 66 73 ATP.
FT CONFLICT 112 112 D -> E (IN REF. 5).
FT TURN 4 4
FT HELIX 5 21
FT HELIX 23 25
FT TURN 29 30
```

etc

etc

b) PIR

```
>P1;RQECA
recA protein - Escherichia coli
C;Species: Escherichia coli
C;Date: 31-Jul-1980 #sequence_revision 14-Nov-1997 #text_change 14-Nov-1997
C;Accession: G65049; A93847; A93846; S11931; S63525; S63979; A03548
...
C;Comment: The recA protein plays an essential role in homologous recombination, in
induction of the SOS response, and in initiation of stable DNA replication.
C;Genetics:
A;Gene: recA
A;Map position: 58 min
C;Superfamily: recA protein
C;Keywords: ATP; DNA binding; DNA recombination; DNA repair; P-loop; SOS response
F;67-75/Region: nucleotide-binding motif A (P-loop)
F;141-145/Region: nucleotide-binding motif B
F;73/Binding site: ATP (Lys) #status predicted
```

Note that these two entries refer to the same gene from E.coli despite differences in the way the data is encoded. However, in contrast to the difference between EMBL and Genbank, the quality of the annotation is quite different. The 3-D structure of this gene has been worked out and this information is reflected in the SwissProt entry as the position of every alpha-helix and beta-sheet is noted. In general, the quality of the annotation and the minimization of internal redundancy makes SwissProt the preferred

database to use. However, note that PIR records the Genetic Map position of the gene; so it is probably good to scrutinize both databases to abstract maximal information.
SwissProt also gives added value by incorporating a large number of DR (database reference) tags, pointing to equivalent information in other databases.

a) SwissProt:

```
DR EMBL; V00328; G42673; -.
DR EMBL; X55553; -; NOT_ANNOTATED_CDS.
DR EMBL; AE000354; G1789051; -.
DR EMBL; D90892; G1800085; -.
DR PIR; A03548; RQECA.
DR PIR; S11931; S11931.
DR PDB; 1REA; 31-OCT-93.
DR PDB; 2REB; 31-OCT-93.
DR PDB; 2REC; 01-APR-97.
DR PDB; 1AA3; 23-JUL-97.
DR SWISS-2DPAGE; P03017; COLI.
DR ECO2DBASE; C039.3; 6TH EDITION.
DR ECOGENE; EG10823; RECA.
DR PROSITE; PS00321; RECA; 1.
DR PFAM; PF00154; recA; 1.
```

When these are used as hypertext links they can enable a WWW browser to locate an extraordinary depth of detail about a given entry, 3-D structure (PDB), protein motifs (Prosite), families of related genes (Pfam), the DNA sequence (EMBL) and a couple of specialist E.coli added-value databases. SRS is one program that makes these hypertext links. The PIR cross-references are far fewer and less explicit; its reference to Genbank (GB:U00096) refers to the whole E.coli genome, whereas SwissProt points specifically to the gene (DR EMBL; V00328)

b) PIR

```
...
A;Cross-references: GB:AE000354; GB:U00096; NID:g2367149; PID:g1789051; UWGP:b2699
```

All these databases are made up of entries, concatenated one after the other in plain readable text. As such they are far bigger than necessary if you are trying to analyze the sequence rather than interrogate or browse the annotation. For these purposes, special high-compressed databases can be constructed. Frequently these are not readable by humans because they have been optimized for speed reading computers.
One of the simplest compression protocols is called Fasta format in which the annotation is edited down to a single title line followed by the sequence. The sequence at the top of the chapter is in Fasta format. All protein databases use the one-letter amino acid code, can you think why this might be?

Sequence Related Databases

Not all biologically relevant Databases consist of sequences and annotation. There are databases of journal abstracts, taxonomy, 3-D structures, mutations and metabolic pathways. Some of the most useful of these are databases which specialise in particular entities that can be found dispersed in the "whole sequence" databases.

You notice one of the cross-references for the SwissProt entry is:

DR PROSITE; PS00321; RECA; 1.

Prosite is a database of protein motifs. PS00321 is a family of proteins that all have the motif:
PA A-L-K-F-[FY]-[STA]-[STAD]-[VM]-R

and are all believed to bind DNA, hydrolyze ATP and act as a recombinase. One of the members of this family is the recA gene in E.coli which gives its name to PS00321. In the pattern above, the residues within [square brackets] are alternatives. Convince yourself that ALKFFAAVR could belong to the family but ALKFAAAVR could not. There are more than 1000 other families classified in a similar way. Finding a Prosite link in a SwissProt gene is a great help in finding other proteins related by structure and/or function.

Interpro - http://www.ebi.ac.uk/interpro/

You should also be aware of the Interpro project which incorporates and sorts data from a diversity of protein motif and domain databases into one searchable meta-database.

Sequence formats

As we have seen comparing database entries above, there are dozens of different ways in which you can store or represent the same fundamental information. Databases are often compiled in, highly conventionalized, readable English text. Computers, being not so bright, will have difficulty reading and interpreting the information unless the conventions are quite rigidly obeyed. There are a very large number of ways you can write, store and transmit simple one-dimensional sequence files. A common sequence interchange program called 'readseq' recognizes at least 22 different file formats. If a computer program does not recognize the format of an input sequence it may not work or, worse, misinterpret header lines as sequence data or otherwise mangle your analysis. The EMBOSS package can also convert between different sequence formats.

EMBOSS

seqret
Reads and writes (returns) sequences in different formats. It can also read in a sequence from a database and write it to a file.

Some commonly used file/sequence formats are shown below:

1) Fasta (named for a widely used homology searching program) – single title line beginning >:

```
>ECRGCG TRANSLATE of: ecrgcg 1 to: 1062
MAIDENKQKALAAALGQIEK
ALGAGGLPMGRIVEIYGPES
TPKAEIEGE*
```

2) Staden (named after Rodger Staden - early, but still extant, software writer) – same as raw sequence:

```
MAIDENKQKALAAALGQIEK
ALGAGGLPMGRIVEIYGPES
TPKAEIEGE*
```

3) NBRF/PIR (named after the protein database):

```
>P1;ecrgcg.pep
ecrgcg.pep, 354 bases, 218 checksum.
MAIDENKQKA LAAALGQIEK
ALGAGGLPMG RIVEIYGPES
TPKAEIEGE*
```

Accession numbers

The information above makes you aware of the diversity of ways in which something so simple as a one-dimensional sequence may be represented. Another source of confusion is the variety of identifying numbers attached to sequences and knowing to which database they refer. Accession numbers are used as unique and unchanging numbers. They are not mnemonic, although databases also have a less stable, more memorable nomenclature: HBB_HUMAN, HSHBB, HUMHBB 2HBB are all human beta globin IDs in various databases,

- **GenBank/EMBL** accession numbers: originally a letter followed by 5 digits (X32152, M22239). When the number of sequences exceeded 2,600,000 - 2 letters followed by 6 digits (AL234556, BF345788).
- **SwissProt**. Still one letter followed by 5 digits, letter is either O,P,Q. P23445.
- **PIR**: the ‘other’ protein database, one letter followed by 5 digits, but numbers confusable with EMBL/GenBank: B93303 is chimp haemoglobin in PIR but a random genomic clone fragment in EMBL.
- **GenPept**. Conceptual translations from DNA that have not yet been annotated well enough to get into SwissProt. three letters and five digits, e.g.: AAA12345.
- **Trembl** (Translated EMBL): O, P or Q followed by 5 letters/digits.
- **PDB** protein structure records: 1 digit and three letters 1HBA, 1TUP

More recently, an attempt has been made to reduce the redundancy in the databases (there were 180 copies of D. melanogaster alcohol dehydrogenase each with its own accession number). One result is RefSeq - NCBI's "reference sequence" database

RefSeq: Two letters, and underscore bar, and six digits,
mRNA records (NM_*) NM_000492 **genomic DNA contigs** (NT_*) NT_000347 **curated/annotated Genomic regions** (NG_*) NG_000567 **Protein sequence records** (NP_*) NP_000483

We will see how RefSeq is becoming the central resource for gene characterization, expression studies, and polymorphism discovery. Because of the high level of necessary curation, it is not anywhere close to being comprehensive even for those species that are included.

Accession numbers give the community a unique label to attach to a biological entity, so we all know we are talking about the same thing. Sequences in databases evolve as their real biological counterparts do. They need to be updated, corrected and merged and we need to know which version of the sequence entry is being referred to. GenBank has used gi numbers and, more recently, version numbers for this. Each small change made to a Genbank record gets the next gi number e.g. gi6995995 and so is totally arbitrary. Version numbers are appended to the accession number after a dot – V00234.2, NM_000492.2.

Interrogating (sequence) databases

SRS - http://srs.ebi.ac.uk/

The DNA databases are enormously rich information resources partly because they are so big, but it would make little sense if it consisted of a long list of As Ts Cs and Gs. There are millions of individual entries in EMBL. An entry could be a fragment as short as 3 base pairs (e.g. M23994) or a large contig consisting of many genes, including complete eukaryotic chromosomes (e.g. X59720). The value of the database lies substantially in the quality of the annotation that puts the sequence in its biological context.

As a biologist you may need to be able to interrogate the Database to find particular sequences or a set of sequences matching given criteria, such as:

The sequence published in Cell 31: 375-382
All sequences from Aspergillus nidulans
Sequences submitted by Peter Arctander
Flagellin or fibrinogen sequences
The glutamine synthase gene from Haemophilus influenzae
The upstream control region of Bacillus subtilis Spo0A

SRS (Sequence Retrieval System) is a very powerful, WWW-based tool, developed by Thure Etzold at EMBL and subsequently managed by Lion Biosciences, for interrogating databases and abstracting information from them.

One of the neatest features of SRS is the fact that interrelated databases can be cross-referenced with WWW hypertext links. This means that you can discover the protein sequence, the cognate DNA sequence, a family of related proteins in other species, a Medline reference to read an abstract of the original publication, a 3-D structure - all with a few point-and-clicks with the mouse.

There are several SRS servers on the Web. We will be using

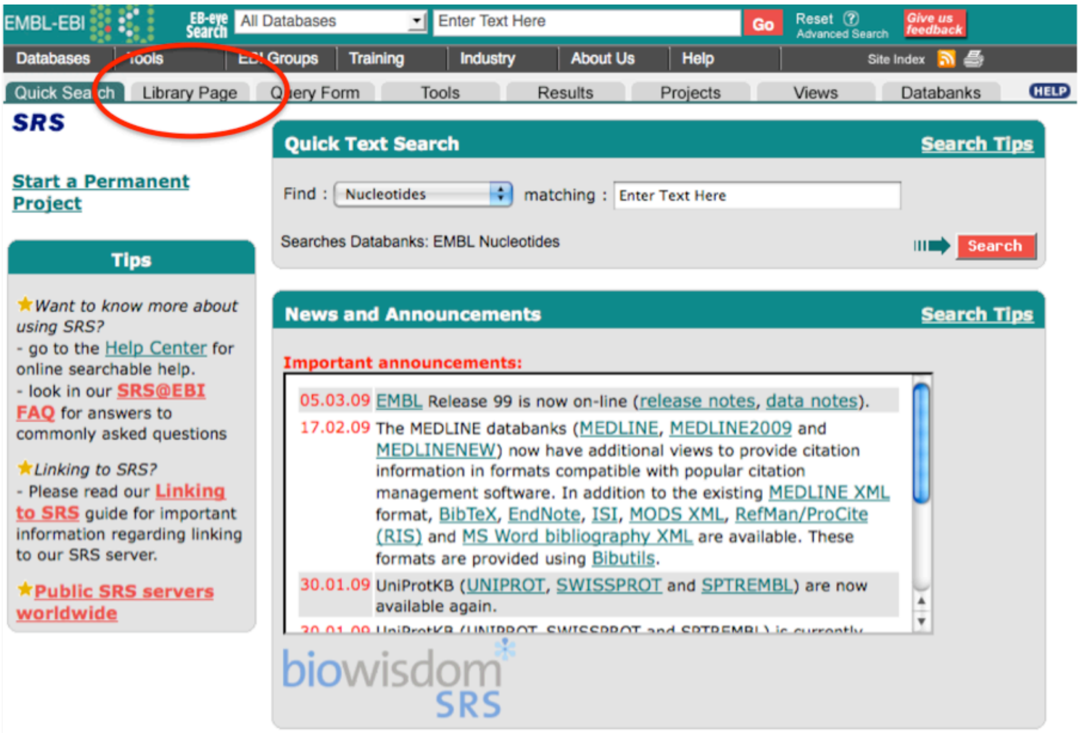
http://srs.ebi.ac.uk/

at the EBI in England because a) it has a large number of interlinked databases b) connectivity to the UK is good c) they are attempting to interconnect their SRS server with their clustalW server and blast server.

With experience and practice you will get to use as much of SRS's power as necessary to obtain the results you need. Below, as a worked example, a series of instructions to obtain the sequences of serum resistance associated proteins in *Trypanosoma brucei* in SwissProt, and download them locally to carry out a multiple sequence alignment using, say, clustalW. It should also be possible to do the multiple alignments on the EBI clustalW server.

Use your browser (Netscape?) to go to http://srs.ebi.ac.uk/ or one of the other SRS servers at the top of the Course page. You should see the following options:

Click on ‘**Library Page**’.


EMBL-EBI
EB-eye Search
All Databases
Enter Text Here
Go
Reset
Advanced Search
Give us feedback
Databases
Tools
EBI Groups
Training
Industry
About Us
Help
Site Index
Quick Search
Library Page
Query Form
Tools
Results
Projects
Views
Databanks
HELP
SRS
Start a Permanent Project
Tips
Quick Text Search
Search Tips
Find :
Nucleotides
matching :
Enter Text Here
Search
News and Announcements
Search Tips
biowisdom SRS

Searches Databanks: EMBL Nucleotides

Want to know more about using SRS?
- go to the Help Center for online searchable help.
- look in our SRS@EBI FAQ for answers to commonly asked questions

Linking to SRS?
- Please read our Linking to SRS guide for important information regarding linking to our SRS server.

Public SRS servers worldwide

Important announcements:

05.03.09 EMBL Release 99 is now on-line (release notes, data notes).

17.02.09 The MEDLINE databanks (MEDLINE, MEDLINE2009 and MEDLINENEW) now have additional views to provide citation information in formats compatible with popular citation management software. In addition to the existing MEDLINE XML format, BibTeX, EndNote, ISI, MODS XML, RefMan/ProCite (RIS) and MS Word bibliography XML are available. These formats are provided using Bibutils.

30.01.09 UniProtKB (UNIPROT, SWISSPROT and SPTREMBL) are now available again.

30.01.09 UniProtKB (UNIPROT, SWISSPROT and SPTREMBL) is currently

This takes you to what is called the TOP PAGE. This page allows you to choose the database(s) that you wish to search. The databases may be of various types, including:

Sequence: Swissprot, sptrembl, PIR (Protein) or EMBL, emblnew (DNA)

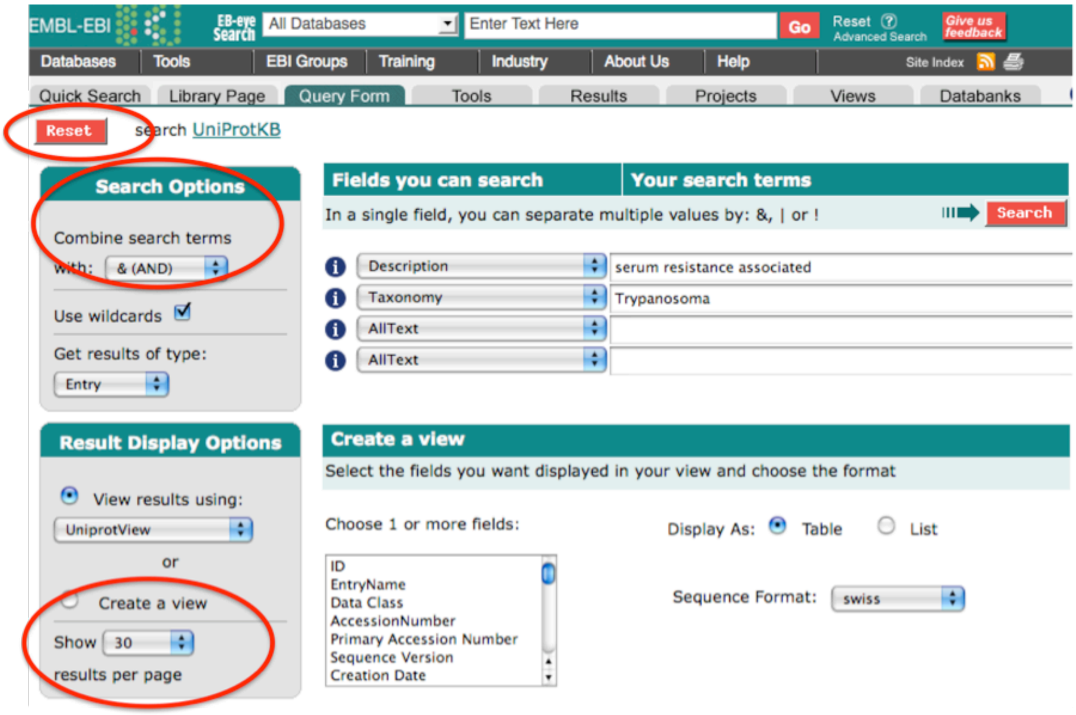
Sequence related: prosite, blocks, prints (protein motifs and alignments), repbase (restriction enzymes),

Protein3Dstructure: PDB, HSSP

For more information about the contents of the database click on the relevant blue underlined hypertext link - UniProt say.

- **Click the box [_] to the left of UniProtKB.**
- **Click on the Query Form tab at the top of the page**

This will move you to a Query Form Page that permits you to submit particular queries (such as have been suggested at the beginning of this chapter) to the databases. At the top of this page will be a note of which database(s) you have chosen to search and a block of four text-insert boxes which you can use to enter your question.


EMBL-EBI
EB-eye Search
All Databases
Enter Text Here
Go
Reset Advanced Search
Give us feedback
Databases
Tools
EBI Groups
Training
Industry
About Us
Help
Site Index
Quick Search
Library Page
Query Form
Tools
Results
Projects
Views
Databanks
Reset
search UniProtKB
Search Options
Result Display Options
Fields you can search
Your search terms
Search
Create a view
Choose 1 or more fields:
ID
EntryName
Data Class
AccessionNumber
Primary Accession Number
Sequence Version
Creation Date
or

Combine search terms with: & (AND)

Use wildcards ☑

Get results of type: Entry

● View results using: UniprotView

○ Create a view

Show 30 results per page

In a single field, you can separate multiple values by: &, | or !

Description: serum resistance associated

Taxonomy: Trypanosoma

AllText:

AllText:

Select the fields you want displayed in your view and choose the format

Display As: ● Table ○ List

Sequence Format: swiss

to the left you will see some things you can change including:

1. **[Reset]** - which clears the screen.
2. **combine search terms &(AND)** - which enables you to apply other logical (boolean) operators.
3. **Use wildcards** which means that "bact" will be interpreted as bact* and look for bacteria, bacteriophage, etc.
4. **Number of entries to display per page** (default is 30)

Your question can be entered into one of more of the text-insert boxes, thus:

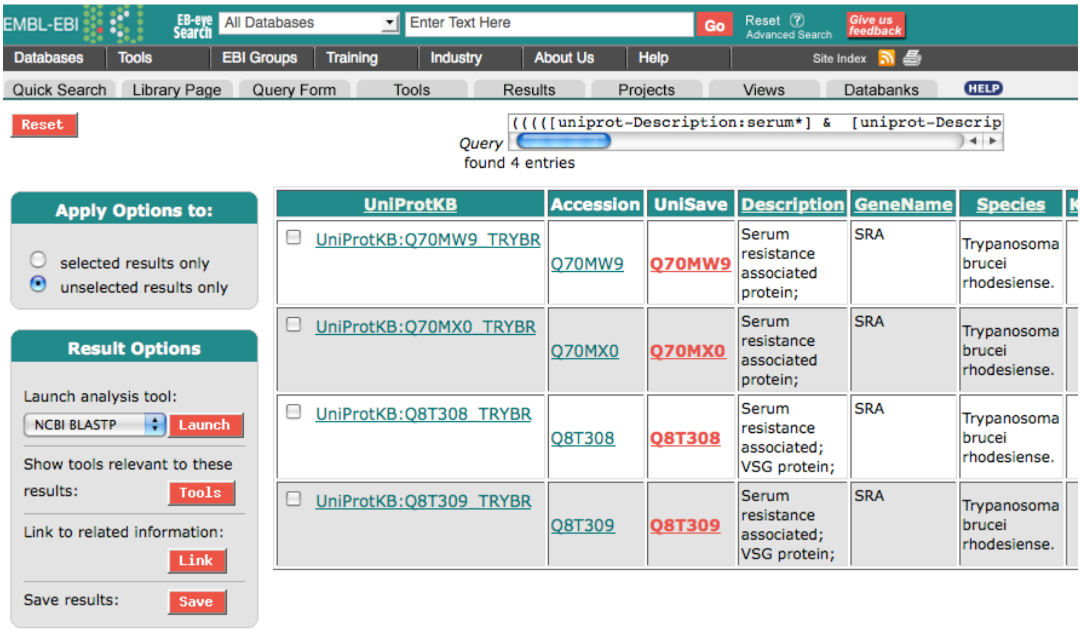
- **Click [All text] change to [Description]** and **insert "serum resistance associated" in box**

Note: it does not have to be "serum resistance associated" it could be ubiquitin or haemoglobin or hemoglobin or actin & alpha. Separate keywords in the same box have to be linked by a logical (Boolean) operator such as

and: &
or: |
but not: !

- **Click the next [All text] change to [Taxonomy] and insert "Trypanosoma" in box**
- **Click Search**

a new window appears with Query "([uniprot-Description: serum resistance associated*] & [uniprot-Taxonomy:Trypanosoma*] " found 4 entries. This is how SRS interprets what you have entered in the boxes and the numbers of "hits" found.


EMBL-EBI
EB-eye Search
All Databases
Enter Text Here
Go
Reset
Advanced Search
Give us feedback
Databases
Tools
EBI Groups
Training
Industry
About Us
Help
Site Index
Quick Search
Library Page
Query Form
Tools
Results
Projects
Views
Databanks
HELP
Reset
Apply Options to:
Result Options
Launch
Tools
Link
Save

Query (((([uniprot-Description:serum*] & [uniprot-Descrip
found 4 entries

○ selected results only
● unselected results only

Launch analysis tool: NCBI BLASTP

Show tools relevant to these results:

Link to related information:

Save results:

UniProtKB	Accession	UniSave	Description	GeneName	Species
☐ UniProtKB:Q70MW9_TRYBR	Q70MW9	Q70MW9	Serum resistance associated protein;	SRA	Trypanosoma brucei rhodesiense.
☐ UniProtKB:Q70MX0_TRYBR	Q70MX0	Q70MX0	Serum resistance associated protein;	SRA	Trypanosoma brucei rhodesiense.
☐ UniProtKB:Q8T308_TRYBR	Q8T308	Q8T308	Serum resistance associated; VSG protein;	SRA	Trypanosoma brucei rhodesiense.
☐ UniProtKB:Q8T309_TRYBR	Q8T309	Q8T309	Serum resistance associated; VSG protein;	SRA	Trypanosoma brucei rhodesiense.

- **Under Display options change [UniprotView] to [FastaSeqs]**
- **Click [Save]**

  Make sure view is [FastaSeqs].
- **Click [Save]**
- **Click Netscape's File ....Save As**
- **Save as type – Text File (*.txt).**
- **Change selection .../wgetz to .../serum.pro and then Click Save.**

This should dump the concatenated fasta format protein sequences into a local file called serum.pro. You can use this file as input for clustalW. There may be local security difficulties with downloading sequences onto a public terminal - check with your neighbours or your demonstrator.

Query manager: a powerful tool

A quick example will show how you can combine very complex queries to zero in on the sequence(s) you need.
Having selected your database(s) go to the Query Form Page and enter:

- **[Description] calmodulin**

you should get about 1140 entries.

- **Click [QUERY] tab** at the top of the page to get a new page and enter:
- **[Organism name] human** (or indeed Homo sapiens)

this will get you a large number of sequences.

- **Click [RESULTS] tab** at the top of the page

A new window should appear with the results for all the queries you have entered in the current SRS session. In the top box of this page **enter "Q1 & Q2"** (leave off the quotes!) **Note**: Your mileage may vary here. Q1 and Q2 may refer to earlier queries in this SRS session (osteonectin?) so use good judgement.
You have just used a boolean logical expression to yield sequences which are a) human and b) have "calmodulin" in the SwissProt description. This shows you how it can be unreliable to depend on the annotation to get homologous sequences. Nevertheless, the list should contain the SwissProt entry for CALM_HUMAN.

Questions

1. Can you think of a better way to find other mammalian calmodulin genes?

2. If you do a search in SwissProt for "calmodulin" using the [AllText] descriptor instead of [Description] you find many more entries, why do you think you get more entries under this search?

3. There are more entries in SwissProt under [Organism] dog than [Author] dog, but more for [Author] wolf than [Organism] wolf. Why do you think this is so?

4. Searching [Organism] mouse in SwissProt yields some plant sequences: prove this by finding sequences matching [Organism] mouse & [Taxon] viridiplantae. Why is this so? (Clue: append wildcard *).

You should be able to reveal the full SwissProt entry for any protein sequence. If you do this you will see several (? blue, underlined) hypertext links to related databases. Almost certainly at least one of these will be EMBL and one to Medline. Probably one will be the prosite motif database. If the 3-D structure is known, one link will be to PDB. Investigate these other databases to get as much relevant information as possible about your sequence.

Aside: Displaying 3-D structures is not "fitted as standard" on all terminals. You may need to get a copy of the RasMol 3-D structure viewer and install it in such a way that your Netscape/IE will recognise it and connect suitable (3-D sequence) file to it.

To display a PDB entry of 3-D coordinates as a rotatable, colorable model you need to click on the **[save]** button. The change the "use mime type" choice-box to **chemical/x-pdb** and then click on the **[save]** box. This should fire up CHIME a WWW implementation of RasMol.) Your mileage may vary!

It is this, interlinked databases, aspect of SRS which gives it a large part of its power. You can extend your search to include other sequences related in some particular (or peculiar!) way. The Prosite link allows you to find members of a protein family. The EMBL link allows you to find the introns and the intron splice junctions, not to mention the ribosome-binding site, the stop codon and the journal reference for the original sequence. The Medline link will give you an abstract etc. You will probably find that:

The PubMed server at http://www.ncbi.nlm.nih.gov/Entrez/ is a far better tool for browsing Medline that what is offered with SRS. Especially powerful is its facility for finding [Related entries].

Additional questions:

“Effective researchers know how to find things out”

1. Who submitted the serum amyloid A (SAA) gene sequence for Canis familiaris?
2. What prosite motif defines the recA family of prokaryotic proteins? Which Dublin-based phylogeneticists used multiple-sequence alignment to define this motif?
3. What are the first and last 5 bases in the intron of the yeast actin gene with EMBL accession number V01288?
4. What is the map position of one of the human SAA genes (SwissProt: P02735)? What cross-reference database is most likely to have map position?
5. What mutation at what position causes phenylketonuria (PKU)? (hint: EMBL K03020) but then try SwissProt: P00439.
6. What bases define the ribosome binding site of the Bacteroides fragilis glnA gene? Perhaps start from the E.coli homolog SwissProt: P06711.
7. Why is the name Saarinen associated with life-threatening cardiac arrythmias? (Hint: not because of architectural flaws...try voltage gated potassium channels)
8. Are there more publicly available DNA sequences from Rodents or Prokaryotes? What about protein sequences?
9. Get a sample of mammalian introns. See what common features they have? Think how these common features might help splicing out the introns.

Entrez - http://www.ncbi.nlm.nih.gov/Entrez/

Entrez is the US equivalent of SRS and is available from the NCBI webpage. You will most likely be familiar with Entrez for interrogating Medline, but the same engine can be pointed at DNA and protein databases. It is handy if you are familiar with the Entrez system and you want a sequence whose name or accession number you already know. At the top of the Entrez page change the Search [__] choice box from PubMed to the appropriate sort of database – the available options are listed on the Entrez page. If you want the sequence alone – to paste into some analysis page – change the Display [__] choice box to FASTA then click on [Save] or [Display] depending on whether you want a permanent or transitory copy of you proteins. Entrez has a more complex syntax for less straightforward queries.

Nucleic Acid Sequence Analysis

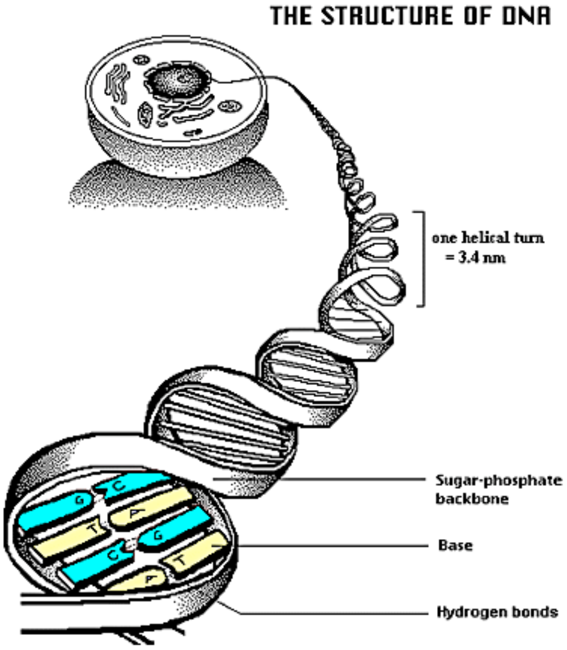
TOPICS:

1. Nucleic acids and the genetic code
2. Translating DNA in 6 frames.
3. Reverse complement & other tools.
4. Calculating some properties of DNA/RNA sequences.
5. Primer design.

1) Nucleic acids and the genetic code

Nucleic acids may be in the form of Deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules containing the genetic information important for all cellular functions and heredity.

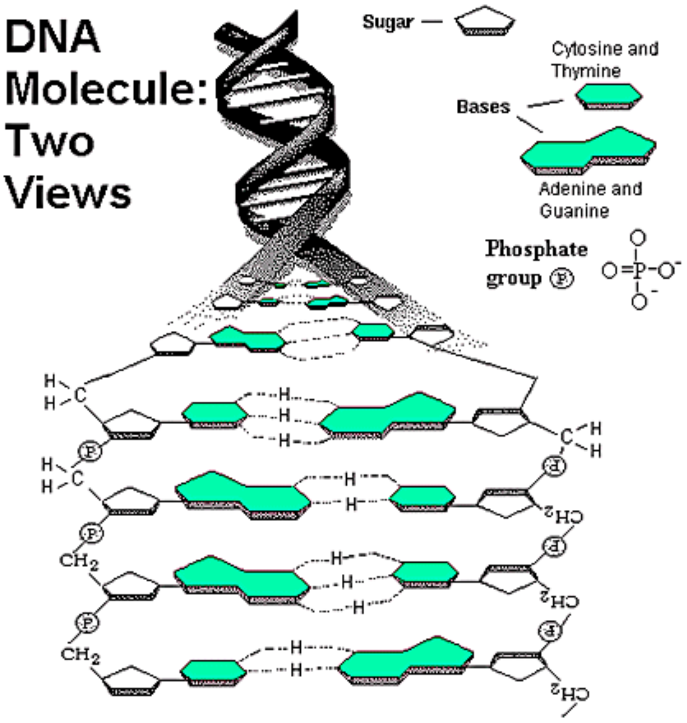
DNA is a long polymer of nucleotides to code for the sequence of amino acid during protein synthesis. DNA is said to carry the genetic ‘blueprint’ since it contains the instructions or information (called genes) needed to construct cellular components like proteins and RNA molecules.



DNA is composed of two strands that twist together to form a helix. Each strand consists of alternating nucleotides. Each nucleotide consists of a phosphate (PO4) and pentose sugar (2-deoxyribose), and attached on the sugar is a nitrogenous base, which can be adenine, thymine, guanine, or cytosine. The four nucleotides are given one letter abbreviations as shorthand for the four bases.

* A is for adenine
* G is for guanine
* C is for cytosine
* T is for thymine

See Appendix1 for more details.



Hence, DNA is a ladder-like helical structure. The two DNA strands are joined together at the center by pairing bases lined up with one another. Adenine pairs with thymine and guanine with cytosine. A and T are connected by two hydrogen bonds. G and C are connected by three hydrogen bonds. DNA is often described structurally as a twisting ladder. In this ladder, the "rungs" are the pairs of bases linked together, and the "sides" are the two separate sugar and phosphate backbones.

The double helix is important because it preserves all of the information-carrying features of a single DNA strand while at the same time introducing elements that make it easier for living cells to make copies of their DNA. Because every base pair in the double helix must match its pairing partner (A with T, C with G), we can easily determine the sequence of an unknown strand of DNA if its matching strand is known. For example, if one strand of a double helix has the nucleotide sequence

```
GATTCGTACG
```

then its complementary strand will be

```
CTAAGCATGC
```

forming a double helix

```
GATTCGTACG
||||||||||
CTAAGCATGC
```

2) Translating DNA in 6-frames:

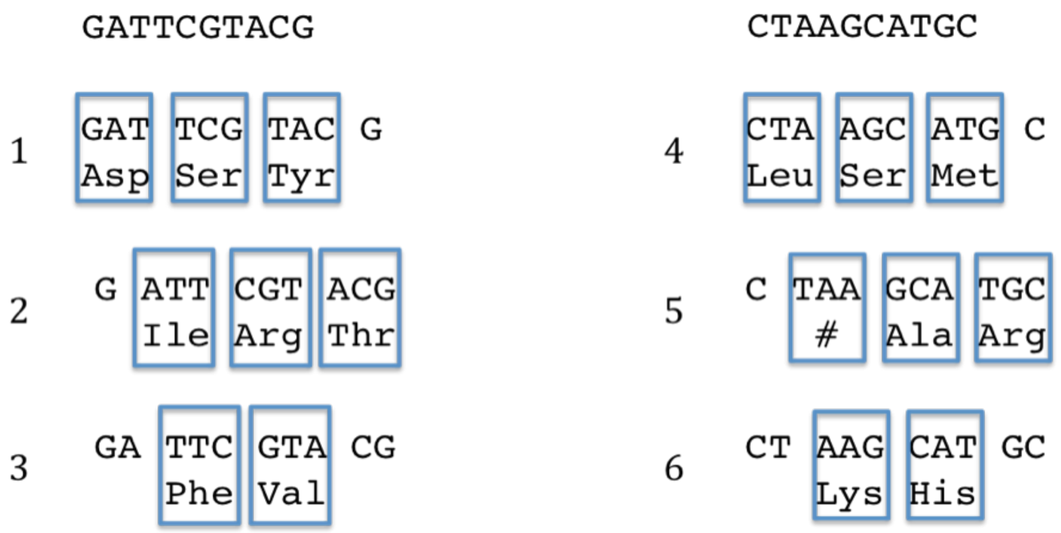
Why six frames?

DNA code for amino acids using a Three-Letter genetic code. (See Appendix II for the complete genetic code.) Since we do not know where to start reading a DNA sequence, we need to look at six different options.

For example the sequence:

```
GATTCGTACG
||||||||||
CTAAGCATGC
```

Can be translated into six different amino acid strings. Looking at each strand separately:


GATTCGTACG
CTAAGCATGC
1
GAT Asp
TCG Ser
TAC Tyr
G
2
G
ATT Ile
CGT Arg
ACG Thr
3
GA
TTC Phe
GTA Val
CG
4
CTA Leu
AGC Ser
ATG Met
C
5
C
TAA #
GCA Ala
TGC Arg
6
CT
AAG Lys
CAT His
GC

Translate tool - http://www.expasy.ch/tools/dna.html

This tool allows the 6-frame translation of a nucleotide (DNA/RNA) sequence to a protein sequence in order to locate open reading frames in your sequence.

- Go to URL above.
- You can use the following phosphoglycerate kinase gene sequence from *Trypanosoma brucei* below or select from here:

```
>Tb927.1.700 phosphoglycerate kinase Trypanosoma brucei
ATGACCCTTA ACGAGAAGAA GAGCATTAAT GAATGCGATC TTAAGGGAAA GAAGGTTCTT
```

```
ATCCGTGTTG ACTTTAATGT TCCCGTGAAA AACGGTAAGA TCACCAACGA CTACCGAATC
CGATCAGCTC TGCCAACGCT CAAGAAGGTT CTCACAGAAG GCGGCAGTTG TGTTCTCATG
AGCCACCTCG GGAGGCCGAA AGGTATTCCC ATGGCGCAAG CTGACAAAAT ACGGAGCACT
GGCGGTGTTC CCGGGTTCCA ACAGAAGGCA ACACTCAAAC CGGTAGCCAA GCGCCTCAGC
GAACTGCTAT TGAGGCCCGT CACATTCGCA CCTGACTGCC TGAATGCTGC AGATGTCGTC
TCTAAGATGT CTCCGGGCGA TGTTGTTCTG CTTGAAAATG TACGCTTTTA CAAAGAAGAG
GGCAGCAAGA AGGCAAAAGA ACGTGAAGCC ATGGCCAAGA TCCTTGCGTC ATATGGTGAT
GTTTACATCA GTGATGCTTT TGGTACAGCT CACCGTGACA GTGCTACCAT GACCGGAATT
CCAAAGATTT TGGGCAACGG TGCTGCCGGT TATTTGATGG AGAAGGAGAT TTCATACTTC
GCTAAGGTAC TTGGTAACCC GCCGCGTCCG CTGGTTGCTA TCGTTGGTGG AGCGAAAGTG
AGCGACAAGA TCCAACTTCT GGATAACATG TTGCAGCGCA TCGATTATCT CTTAATTGGT
GGTGCAATGG CATACACATT TCTGAAGGCT CAGGGTTACA GCATTGGAAA ATCGAAGTGC
GAGGAAAGTA AACTTGAATT TGCTCGATCC CTGCTGAAGA AGGCGGAGGA CCGCAAGGTG
CAGGTTATTC TTCCAATTGA TCATGTTTGC CACACGGAAT TCAAAGCTGT GGATTCTCCA
TTGATAACTG AGGATCAAAA CATCCCTGAA GGACATATGG CTCTGGATAT TGGTCCCAAG
ACTATTGAAA AATATGTTCA GACGATTGGG AAGTGTAAGA GCGCCATTTG GAACGGTCCC
ATGGGTGTAT TTGAAATGGT TCCTTATTCC AAAGGTACAT TTGCAATTGC GAAAGCCATG
GGTCGAGGAA CTCACGAGCA TGGACTCATG AGTATCATCG GTGGTGGTGA CAGCGCAAGT
GCAGCTGAGT TGAGCGGTGA GGCGAAGCGC ATGTCTCATG TTTCAACTGG TGGTGGTGCG
TCTTTGGAAC TCCTCGAGGG CAAAACGCTT CCCGGCGTTG CAGTATTGGA CGAAAAGTCG
GCGGTTGTGT CGTATGCCTC TGCAGGTACT GGAACTCTTT CTAACCGGTG GAGCTCTCTT
TAA
```

- Paste your sequence in the box provided & click “TRANSLATE SEQUENCE”.
- You can choose 3 options
  - Verbose – puts Met & Stop to highlight start & stop codons.
  - Compact – useful if you want to use output in other programs.
  - Includes nucleotide sequence – nucleotide sequence is above the translation.
- This returns a 6-frame translation of your sequence. You can then choose the correct frame.

EMBOSS

transeq

Translate nucleic acid sequences

3) Reverse Complement & other tools:

There are many cases where you might want to obtain the reverse complement of a DNA sequence, for example the reverse complement is needed as a negative control when doing a DNA hybridisation experiment.

Search launcher at Baylor College –

http://searchlauncher.bcm.tmc.edu/seq-util/seq-util.html

This tool contains a number of different applications for nucleic acid sequence analysis: For each application you can click on the following **[H]** **[O]** **[P]** **[E]** =

[H]:Help/description; [O]:full Options form; [P]:search Parameters; [E]:Example search. On all the Baylor pages (and everywhere else possible) it is important to investigate the options [**O**] to see a) what are the defaults and b) what options seem worth changing. The following programs are available:

- **Readseq:**

Converts nucleic acid/protein sequences between any of 30 different formats. It is often appropriate to convert to FASTA format. A large number of input formats are permitted. See help for details [H].

- **RepeatMasker:**

RepeatMasker is a program that screens DNA sequences for interspersed repeats known to exist in mammalian genomes as well as for low complexity DNA sequences. The output of the program is a detailed annotation of the repeats that are present in the query sequence as well as a modified version of the query sequence in which all the annotated repeats have been masked (replaced by Ns). On average, over 40% of a human genomic DNA sequence is masked by the program. This is important in primer design so that you do not design a primer that spans a region with repeats. It is also important before doing a homology search as repeats in your sequence may hit other repeats in the genome (although BLAST now does this for you).

- **Primer Selection** -PCR primer selection (See primer design later).
- **WebCutter**- restriction maps using enzymes w/ sites >= 6 bases.
- **6 Frame Translation** - translates a nucleic acid sequence in 6 frames.
- **Reverse Complement** - reverse complements a nucleic acid sequence.
- **Reverse Sequence** - reverses sequence order.
- **Sequence Chopover** - cut a large protein/DNA sequence into smaller ones with certain amounts of overlap.
- **HBR -** Finds *E.coli* contamination in human sequences.

EMBOSS

revseq
Reverse and complement a sequence

eprimer3
Picks PCR primers and hybridization oligos

primersearch
Searches DNA sequences for matches with primer pairs

restrict
Finds restriction enzyme cleavage sites

transeq
Translate nucleic acid sequences

prettyseq
Output sequence with translated ranges

plotorf
Plot potential open reading frames

showorf
Pretty output of DNA translations

splitter
Split a sequence into (overlapping) smaller sequences

Exercise: Paste in the phosphoglycerate kinase gene sequence from *Trypanosoma brucei* or alternatively examine an example output for each application by clicking [**E**] beside each program. Pay particular attention to the options available: these will give you clues about standard practice.

See if you can repeat the exercise using the EMBOSS program's.

See Appendix1 and Appendix2 for details about the genetic code.

4) Oligo Calculator - http://www.pitt.edu/~rsup/OligoCalc.html

Tool to calculate the **length**, **%GC content**, **Melting temperature (Tm)** the midpoint of the temperature range at which the nucleic acid strands separate, **Molecular weight**, & what an **OD** = 1 is in picoMolar of your input nucleic acid sequence.

Many of these parameters are useful in primer design (see next section) and in other areas of molecular biology.

- Go to URL above.
- Paste the phosphoglycerate kinase gene sequence from *Trypanosoma brucei* in the box provided and click “**Calculate**”.

Example:

>Tb927.1.700 phosphoglycerate kinase Trypanosoma brucei
Length = 1333
% GC content = 49
Tm = 84 °C
Molecular Weight = 412911 daltons (g/M)
OD of 1 = 68 picoMolar

EMBOSS

dan
Calculates DNA RNA/DNA melting temperature

eprimer3
Picks PCR primers and hybridization oligos

5) Primer design

(Originally written in Jan 2002 by Dr. Norma O’Donovan (Thanks!).)

The recommended site (although there are several others available on the web) is GeneFisher:

http://bibiserv.techfak.uni-bielefeld.de/genefisher/help/wwwgfdoc.html

The submission form:

http://bibiserv.techfak.uni-bielefeld.de/cgi-bin/gf_submit?mode=STARTUP&sample=dna

The input form is straightforward and well documented.

Primer Design Tips:

- **Primer Length:** usually between 18 and 24 base pairs
- **% GC**: Optimum GC content is 45-55%
- **Annealing Temperature:** Should be between 55 ^{o}C and 65 ^{o}C and ideally the annealing temperature of the 2 primers should be similar. A quick equation (Wallace formula) for calculating the annealing temperature of the primer is:

2 x (no. of As + Ts) + 4 x (no. of Gs + Cs)

The lower of the 2 primer annealing temperatures is the highest temperature that can be used for annealing. (Usually when optimising PCR you would start with an annealing temp. a few degrees below the Tm of the primers).

- **G/C clamps:** The 3’ end of the primer should be able to form G/C clamps, i.e. several consecutive G/C or C/G base pairs, between the 3' end of the primer and the template DNA.
- **Length of PCR product:** The optimum size is 100 – 500 base pairs for conventional PCR. Shorter products can be used for real time PCR or longer products can be amplified using special polymerases.

Things to avoid:

1. Complimentarity within a primer or between 2 primers (especially in the ends), used in the same reaction, as this may cause primer dimers.

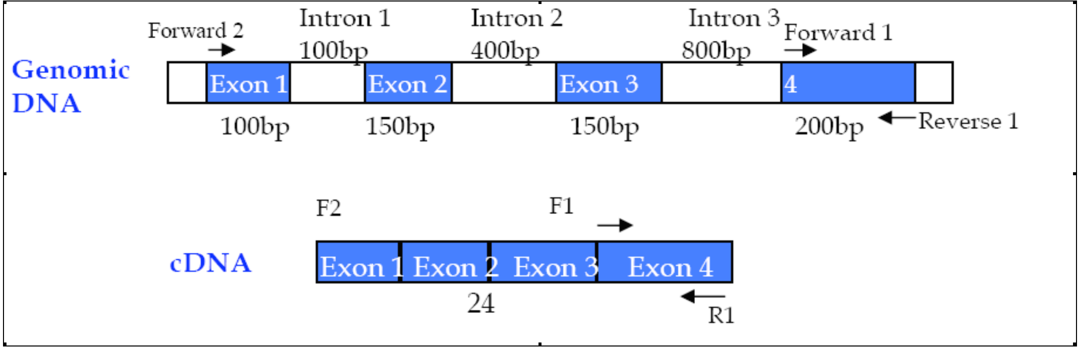
2. Strings of a single nucleotide (more than 3).

3. Non-specific binding of primers to related sequences – check the specificity of the primers by doing a BLAST search of the database (non-redundant and genomic) with each of the primer sequences.

Primers for RT-PCR

The same rules as above apply but there are a few extra considerations. If you are doing RT-PCR with total RNA there may be genomic DNA contamination present in the RNA. (You can DNase treat to remove it or purify poly-A mRNA). If it is not removed you must ensure that your primers specifically amplify the cDNA (complementary to mRNA). Ideally the primers should not amplify the genomic DNA at all but if that is not possible the genomic product should be distinguishable from the cDNA product on a gel, based on size. Therefore the primers must span at least one intron in the genomic DNA. To identify the position of introns in the sequence align the mRNA sequence with the genomic sequence using a pairwise BLAST sequence alignment (http://www.ncbi.nlm.nih.gov/blast). Alternatively, for human or mouse sequences, on the UCSC website (http://genome.ucsc.edu/) you can do a BLAT search with the mRNA which will identify the intron/exon structure of the gene.

Example:


Genomic DNA
Forward 2
Intron 1
100bp
Intron 2
400bp
Intron 3
800bp
Forward 1
Exon 1
Exon 2
Exon 3
4
100bp
150bp
150bp
200bp
Reverse 1
cDNA
F2
F1
Exon 1
Exon 2
Exon 3
Exon 4
24
R1

If the forward and reverse primers are designed in exon 4 the PCR product obtained from the cDNA will be the same size as the genomic PCR product. If the forward primer is in exon 1 and the reverse primer is in exon 4 the cDNA product will be approx. 600bp whereas the PCR product from genomic DNA would be about 1900bp, which probably wouldn’t be amplified in conventional PCR.

EMBOSS

eprimer3
Picks PCR primers and hybridization oligos

Protein Sequence Analysis

TOPICS

1. Physico-chemical properties.
2. Cellular localization.
3. Signal peptides.
4. Transmembrane domains.
5. Post-translational modifications.
6. Motifs & domains.
7. Secondary structure.
8. Other resources.

ExPASy - http://www.expasy.ch/

The ExPASy (**Ex**pert **P**rotein **A**nalysis **Sy**stem) protein and proteomics server of the Swiss Institute of Bioinformatics (SIB) is dedicated to the analysis of protein sequences and structures. Besides the tools that we will introduce in this manual there are many other applications available at this website that you should take some time to have a look at.

1) Physico-chemical properties:

ProtParam tool – http://www.expasy.ch/tools/protparam.html

Calculates lots of physico-chemical parameters of a protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY)

Example: Human BRCA 1

You can paste the gene sequence brca1 from the course website.

- At ExPASy → "Proteomics and sequence analysis tools" → "Primary structure analysis".
- Click on the "ProtParam" link.
- Paste your sequence in the box provided
- The sequence must be written using the one letter amino acid code:
- Press the "Compute parameters" button.
- The output for this sequence is shown below.

```
Number of amino acids: 1863
Molecular weight: 207720.8
Theoretical pI: 5.29
Amino acid composition:
Ala (A) 84 4.5%
Arg (R) 76 4.1%
Etc etc
Thr (T) 111 6.0%
Trp (W) 10 0.5%
Tyr (Y) 31 1.7%
Val (V) 101 5.4%
Asx (B) 0 0.0%
Glx (Z) 0 0.0%
Xaa (X) 0 0.0%
```

Total number of negatively charged residues (Asp + Glu): 283
Total number of positively charged residues (Arg + Lys): 213
Atomic composition:
Carbon C 8908
Hydrogen H 14246
Nitrogen N 2554
Oxygen O 3014
Sulfur S 74
Formula: $C_{8908}H_{14246}N_{2554}O_{3014}S_{74}$
Total number of atoms: 28796
Extinction coefficients:
Conditions: 6.0 M guanidium hydrochloride
0.02 M phosphate buffer
pH 6.5
Extinction coefficients are in units of $M^{-1} cm^{-1}$.
The first table lists values computed assuming ALL Cys residues appear as half cystines, whereas the second table assumes that NONE do.

```
276 278 279 280 282
nm nm nm nm nm
Ext. coefficient 102140 102194 100935 99220 95840
Abs 0.1% (=1 g/l) 0.492 0.492 0.486 0.478 0.461
276 278 279 280 282
nm nm nm nm nm
Ext. coefficient 98950 99400 98295 96580 93200
Abs 0.1% (=1 g/l) 0.476 0.479 0.473 0.465 0.449
```

Estimated half-life:
The N-terminal of the sequence considered is M (Met).
The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).
Instability index:
The instability index (II) is computed to be 54.68
This classifies the protein as unstable.
Aliphatic index: 69.01
Grand average of hydropathicity (GRAVY): -0.785

EMBOSS

pepinfo
Plots simple amino acid properties

pepstats
Protein statistics

charge
Protein charge plot

iep
Calculates the isoelectric point of a protein

2) Cellular localization:

PSORT - http://psort.nibb.ac.jp/form2.html

PSORT, a program to predict the subcellular localization sites of proteins from their amino acid sequences. This program makes use of the fact that proteins destined for particular subcellular localizations have distinct amino acid properties particularly in their N-terminal regions. These properties can be used to predict whether a protein is localized in the cytoplasm, nucleus, mitochondria, or is retained in the ER, or destined for the lysosome (vacuolar) or the peroxisome. There is a detailed page of output that we can probably ignore. At the end of the output the percentage likelihood of the subcellular localization is given. If you want to learn more about the output and how subcellular localization is determined please see the user manual at:

http://psort.nibb.ac.jp/helpwww2.html

Example: Human ETS-1 protein.

You can paste the gene sequence ets-1 from the course website.

- At ExPASy (www.expasy.ch) → "Post-translational modification prediction".
- Click on the "PSORT" link.
- For animal/yeast sequences click the link to "PSORT II Prediction".
- Paste your sequence in the box provided.
- The sequence must be written using the one letter amino acid code:
- Press the submit button.
- The output for this sequence is shown below.

There are a number parameters measured by this program which you can read about as links from the output file. By scrolling to the bottom of the output you can see the probability that this sequence is nuclear, cytoplasmic, peroxisomal, vacuolar or cytoskeletal. PSORT predicts that ETS-1 is nuclear with a high probability. The fact that ETS-1 is localized in the nucleus has been previously experimentally determined.

Results of Subprograms

```
PSG: a new signal peptide prediction method
N-region: length 8; pos.chg 2; neg.chg 1
H-region: length 6; peak value 1.89
PSG score: -2.51
```

Results of the k-NN Prediction

```
k = 9/23
73.9 %: nuclear
13.0 %: cytoplasmic
4.3 %: peroxisomal
4.3 %: vacuolar
4.3 %: cytoskeletal
>> prediction for QUERY is nuc (k=23)
```

3) Signal peptides:

Proteins destined for secretion, operation with the endoplasmic reticulum, lysosomes and many transmembrane proteins are synthesized with leading (N-terminal) 13 – 36 residue signal peptides.

SignalP - http://www.cbs.dtu.dk/services/SignalP/

The SignalP WWW server can be used to predict the presence and location of signal peptide cleavage sites in your proteins. It can be useful to know whether your protein has a signal peptide as it indicates that it may be secreted from the cell. Furthermore, proteins in their active form will have their signal peptides removed, if you can determine the length of the signal peptide then you can calculate the size of the protein minus the signal peptide.

Example: Human Beta-defensin; sp|Q09753|BD01_HUMAN

You can paste the gene sequence HBD1 from the course website.

At ExPASy → "Post-translational modification prediction".

Click on the "SignalP" link.

Paste your sequence in the box provided

The sequence must be written using the one letter amino acid code:

It is recommend that the N-terminal part only (not more than 50-70 amino acids) of the sequences is submitted. A longer sequence will increase the risk of false positives and make the graphical output difficult to read. The new version now automatically truncates input sequences.

Choose one or more group of organisms for the prediction by clicking the check-box next to the group(s):

If no groups are indicated, predictions from all three groups will be returned.

A graphical output (in Postscript format) of the prediction will be available, if the "Include graphics"-button is checked.

Press the "Submit sequence" button.

A WWW page will return the results when the prediction is ready. Response time depends on system load. The output for this sequence is shown below

C score = raw cleavage site score

The output score from networks trained to recognize cleavage sites vs. other sequence positions. Trained to be: High at position +1 after the cleavage site and low at all other positions.

S score = signal peptide score

The output score from networks trained to recognize signal peptide vs. non-signal-peptide positions. Trained to be: High at position before the cleavage site and low at all other positions.

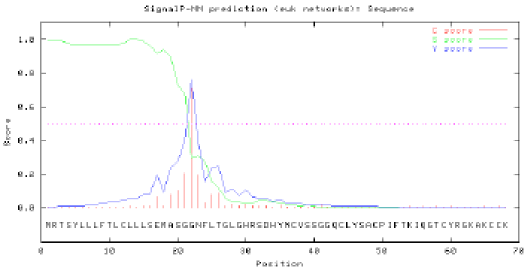
Y score = combined cleavage site score

The prediction of cleavage site location is optimized by observing where the C-score is high and the S-score changes from a high to a low value.

For each sequence, SignalP will report the maximal C, S, and Y scores, and the mean S-score between the N-terminal and the predicted cleavage site. These values are used to distinguish between signal peptides and non-signal peptides. If your sequence is predicted to have a signal peptide, the cleavage site is predicted to be immediately before the position with the maximal Y-score.

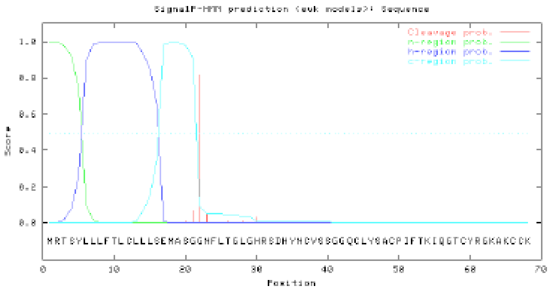
The Human beta-defensin protein has a predicted signal peptide from position 1 to 21 and a potential cleavage site exists between positions 21 and 22. These predictions correspond exactly to the SWISS-PROT annotation for this protein (accession Q09753).

SignalP-NN result:



```
# data
>Sequence length = 68
# Measure Position Value Cutoff signal peptide?
max. C 22 0.710 0.32 YES
max. Y 22 0.761 0.33 YES
max. S 14 0.998 0.87 YES
mean S 1-21 0.943 0.48 YES
D 1-21 0.852 0.43 YES
# Most likely cleavage site between pos. 21 and 22: ASG-GN
```

SignalP-HMM result:



```
# data
>Sequence
Prediction: Signal peptide
Signal peptide probability: 1.000
Signal anchor probability: 0.000
Max cleavage site probability: 0.818 between pos. 21 and 22
```

EMBOSS

sigcleave
Reports protein signal cleavage sites

4) Transmembrane domains:

Tmpred - http://www.ch.embnet.org/software/TMPRED_form.html

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins. The prediction is made using a combination of several weight-matrices for scoring. The presence of transmembrane domains is an indication that the protein is located on the cell surface.

Example: Human chemokine receptor 4 protein sequence NP_003458.1

You can paste the gene sequence chemo4 from the course website.

At ExPASy → “Topology prediction”.

Click on the link to Tmpred.

Paste your sequence in the box provided in one of the supported formats e.g.

plain text, SwissProt_ID or AC, etc.

You may change the minimal and maximal length of the hydrophic part of the transmembrane helix but unless you have reason to do so you should accept the defaults i.e. 17 and 33. ~22 residues is the same length as the width of a lipid bilayer.

Click the “Run Tmpred” button to start the search.

The output is given in 3 parts 1, 2 and 3 (see below).

Part 1: lists all the significant predictions of possible transmembrane helices

in this case there are 7 helices predicted but at this stage we do not know the orientation of the helices so there are 2 tables, the first with the helices orientated from the inside to the outside and vice versa for the second.

Part 2: shows which inside->outside helices correspond to the outside -> inside helices and indicates which orientation is most likely.

Part 3: proposes the strongly preferred model for the transmembrane domain structure of the protein and also an alternative model.

A graphic of the prediction is also available (not shown here)

These predictions correspond well but not exactly to the SWISS-PROT annotation for this protein (accession P30991).

Tmpred output

Sequence: MEG...HSS, length: 352

Prediction parameters: TM-helix length between 17 and 33

---

1. Possible transmembrane helices

The sequence positions in brackets denominate the core region. Only scores above 500 are considered significant.

```
Inside to outside helices : 7 found
from to score center
39 ( 46) 62 ( 62) 1962 54
78 ( 85) 105 ( 103) 1623 95
114 ( 114) 133 ( 130) 1352 122
155 ( 157) 175 ( 173) 1716 165
204 ( 206) 223 ( 223) 2052 214
240 ( 240) 261 ( 259) 2840 251
286 ( 286) 305 ( 305) 1241 295
Outside to inside helices : 7 found
from to score center
47 ( 47) 63 ( 63) 2568 55
78 ( 78) 96 ( 96) 1331 86
111 ( 114) 132 ( 132) 1740 122
```

```
155 ( 157) 173 ( 173) 1197 165
204 ( 204) 223 ( 223) 2404 214
240 ( 242) 259 ( 259) 2037 251
283 ( 286) 305 ( 305) 1703 294
```

---

2. Table of correspondences

Here is shown, which of the inside->outside helices correspond to which of the outside->inside helices.
Helices shown in brackets are considered insignificant. A “+”-symbol indicates a preference of this orientation. A “++”-symbol indicates a strong preference of this orientation.

```
Inside->outside | outside->inside
39- 62 (24) 1962 | 47- 63 (17) 2568 ++
78- 105 (28) 1623 ++ | 78- 96 (19) 1331
114- 133 (20) 1352 | 111- 132 (22) 1740 ++
155- 175 (21) 1716 ++ | 155- 173 (19) 1197
204- 223 (20) 2052 | 204- 223 (20) 2404 ++
240- 261 (22) 2840 ++ | 240- 259 (20) 2037
286- 305 (20) 1241 | 283- 305 (23) 1703 ++
```

---

3. Suggested models for transmembrane topology

These suggestions are purely speculative and should be used with extreme caution since they are based on the assumption that all transmembrane helices have been found. In most cases, the Correspondence Table shown above or the prediction plot that is also created should be used for the topology assignment of unknown proteins.

```
2 possible models considered, only significant TM-segments used
---→ STRONGLY preferred model: N-terminus outside
7 strong transmembrane helices, total score : 14594
# from to length score orientation
1 47 63 (17) 2568 o-I
2 78 105 (28) 1623 I-o
3 111 132 (22) 1740 o-I
4 155 175 (21) 1716 I-o
5 204 223 (20) 2404 o-I
6 240 261 (22) 2840 I-o
7 283 305 (23) 1703 o-I
----→ alternative model
7 strong transmembrane helices, total score : 11172
# from to length score orientation
1 39 62 (24) 1962 I-o
2 78 96 (19) 1331 o-I
3 114 133 (20) 1352 I-o
4 155 173 (19) 1197 o-I
5 204 223 (20) 2052 I-o
6 240 259 (20) 2037 o-I
7 286 305 (20) 1241 I-o
```

EMBOSS

tmap
Displays membrane spanning regions

5) Post-translational modifications:

After translation has occurred proteins may undergo a number of posttranslational modifications. These can include the cleavage of the pro- region to release the active protein, the removal of the signal peptide and numerous covalent modifications such as, acetylations, glycosylations, hydroxylations, methylations and phosphorylations. Posttranslational modifications such as these may alter the molecular weight of your protein and thus its position on a gel. There are many programs available for predicting the presence of posttranslational modifications, we will take a look at one for the prediction of type O-glycosylation sites in mammalian proteins. Remember these programs work by looking for consensus sites and just because a site is found does not mean that a modification definitely occurs.

NetOGlyc - http://www.tigr.org/db.shtml

Prediction of type O-glycosylation sites in mammalian proteins. This program works by comparing the input sequence to a database of known and verified mucin type O-glycosylation sites extracted from O-GLYCBASE.

Example: Human CD1D sp|P15813|CD1D_HUMAN

You can paste the gene sequence cd1d from the course website.

- At ExPASy → "Post-translational modification".
- Click on the link to "NetOGlyc".
- Paste your sequence in the box provided in FASTA format.
- Check "generate graphics" and click the submit button.
- The output for this program is shown below (graphics not shown).
- This program predicts potential O-glycosylation sites at Threonine 64 and Serine 214.

NetOGlyc 2.0 Prediction Results

```
Name: Sequence Length: 335
MGCLLFLLLWALLQAWGSAEVPQRLFPLRCLQISSFANSSWTRTDGLAWLGELQTHSWSNDSDTVRSLKPWSQGTFSDQQ 80
WETLQHIFRVYRSSFTRDVKEFAKMLRLSYPLELQVSAGCEVHPGNASNNFFHVAFQGKDILSFQGTSWEPTQEAPLWVN 160
LAIQVLNQDKWTRETVQWLLNGTCPQFVSGLLESGKSELKKQVKPKAWLSRGPSPGPGRLLLVCHVSGFYPKPVWVKWMR 240
GEQEQQGTQPGDILPNADETWYLRATLDVVAGEAAGLSCRVKHSSLEGQDIVLYWGGSYTSMGLIALAVLACLLFLLIVG 320
FTSRFKRQTSYQGVL
...............................................................T................ 80
................................................................................ 160
.....................................................S.......................... 240
................................................................................ 320
...............

Name Residue No. Potential Threshold Assignment
Sequence Thr 42 0.0611 0.6493 .
Sequence Thr 44 0.0087 0.6573 .
Sequence Thr 55 0.0117 0.6491 .
Etc etc
Sequence Thr 248 0.0131 0.5840 .
Sequence Thr 260 0.0089 0.6578 .
Sequence Thr 266 0.0224 0.6957 .
Sequence Thr 300 0.0147 0.7357 .
Sequence Thr 322 0.0480 0.7096 .
Sequence Thr 329 0.0639 0.6021 .

Name Residue No. Potential Threshold Assignment
Sequence Ser 18 0.0161 0.6211 .
Sequence Ser 34 0.0044 0.6673 .
Sequence Ser 35 0.0048 0.6717 .
Sequence Ser 39 0.0337 0.6118 .
Sequence Ser 40 0.0013 0.6521 .
Sequence Ser 57 0.0065 0.6484 .
Etc etc
Sequence Ser 284 0.0005 0.6401 .
Sequence Ser 285 0.0082 0.6389 .
Sequence Ser 298 0.0003 0.6778 .
Sequence Ser 301 0.0007 0.6924 .
Sequence Ser 323 0.0003 0.6441 .
Sequence Ser 330 0.0052 0.6277 .
```

Note: The new version of this server does not predict these sites. This is a good lesson in the evolving nature of these servers and why validation at more than one is a good idea.

6) Motifs and Domains

If you want to determine the function of a protein the first tool of choice is homology searching (see day 4). Unless this finds you a match with a well characterized protein comprehending the entire length of yours you should look for motifs and domains in your protein. To determine if your protein sequence contains known motifs or conserved domain structures you should search the protein against one of the motif or profile databases. There are many of these available but we will discuss ProfileScan (now called myHits), which allows you to search both the Prosite and Pfam databases simultaneously. See the documentation for more details.

ProfileScan - http://hits.isb-sib.ch/cgi-bin/PFSCAN

Example: Human CFTR sp|P13569|CFTR_HUMAN

You can paste the gene sequence cftr from the course website.

- Go to the URL above
- Paste your sequence in the box provided.
- The sequence must be written using the one letter amino acid code:
- Tick the motif databases you wish to search, other parameters should be OK.
- Press the “scan” button.

The output for this program is too large to show here, but it gives lots of detail about motifs in the CFTR protein identifying potential: ABC transporters family signature; ATP/GTP-binding site motif A (P-loop); Protein kinase C phosphorylation sites; N-glycosylation sites; Casein kinase II phosphorylation site; N-myristoylation sites; cAMP- and cGMP-dependent protein kinase phosphorylation site; Bipartite nuclear localization signal; NACHT-NTPase domain profile; Guanylate kinase domain profile etc.

Remember that these programs only tell you are that there is a motif present and thus there is the potential for these modifications and functions to occur. It is up to you to determine experimentally which are real but at least you now know what to look for.

7) Secondary Structure Prediction

If protein structure, even secondary structure, can be accurately predicted from the now abundantly available gene and protein sequences, such sequences become immensely more valuable for the understanding of drug-design, the genetic basis of disease, the role of protein structure in its enzymatic, structural, and signal transduction functions, and basic physiology from molecular to cellular, to fully systemic levels. In short, the solution of the protein structure prediction problem (and the related protein folding problem) will bring on the second phase of the molecular biology revolution (Munson et al., 1994).

JPRED – http://www.compbio.dundee.ac.uk/www-jpred/

Jpred is an Internet web server that takes either a protein sequence or a multiple alignment of protein sequences, and predicts secondary structure. It works by combining a number of modern, high quality prediction methods to form a consensus. Please be aware that secondary structure prediction is an extremely complex problem that is under intensive research and we are still at a relatively primitive stage. We cannot discuss the details of protein secondary structure here but if you are interested in this area we recommend that you take a look at any major biochemistry textbook. Essentially protein secondary structure consists of 3 major conformations; the α Helix, the β pleated sheet and the coil conformation.

Example: Human alpha 1 hemoglobin. NP_000549.1

You can paste the gene sequence hbb from the course website.

- At the ExPASy → “Secondary structure prediction”.
- Click on the link to JPRED.
- Click “Prediction”.
- Paste your sequence in the box provided.
- The defaults are OK.
- Click “Run secondary structure predictions!”
- Point 4 on the submission page allows you to deselect the BLAST search against PDB (Protein Data Bank). If your sequence already has had its structure predicted or experimentally determined it will be in here and you can follow the link to PDB for information on the structure of your protein.
- If your protein is in PDB you can view your protein secondary structure using RasMol (To download RasMol see the course website for a link).

- Once you have RasMol running you can open your structure in it a view it using a number of different options.

Otherwise continue with prediction

- The program may take a long time so you can save a bookmark and return to your results later or choose to have your results e-mailed to you.
- There are a number of options to view the output, view your output in HTML format (option 4).
- The complete output is too large to show here (see webpage).
- Scroll down through the output until you get to "Jpred" output. The line of output beside this is the consensus secondary structure for your sequence. H= Helices E= strands C= coils.

A Few Other Useful Tools at ExPASy

www.expasy.ch

FindMod

Predicts potential protein post-translational modifications (PTM) and find potential single amino acid substitutions in peptides. The experimentally measured peptide masses are compared with the theoretical peptides calculated from a specified SWISS-PROT/TrEMBL entry or from a user-entered sequence, and mass differences are used to better characterise the protein of interest.

NetPhos:

The NetPhos WWW server produces neural network predictions for serine, threonine and tyrosine phosphorylation sites in eukaryotic proteins.

Sulfinator:

Predicts tyrosine sulfation sites in protein sequences. Tyrosine sulfation is an important post-translational modification of proteins that go through the secretory pathway.

REP:

Searches a protein sequence for a collection of repeats such as leucine rich repeats and many others.

Other Resources for Protein Sequence Analysis

1) Protein Prospector at UCSF - http://prospector.ucsf.edu/

MS-Digest: A protein digestion tool from the UCSF Mass Spectrometry Facility that performs an in silico enzymatic digestion of a protein sequence, and calculates the mass of each peptide.

MS-Product: A tool from the UCSF Mass Spectrometry Facility that calculates the possible fragment ions resulting from fragmentation of a peptide in a mass spectrometer. Fragmentation possibilities for post-source decay (PSD), high-energy collision-induced dissociation (CID), and low-energy CID processes may be calculated.

2) Pasteur Institute - http://bioweb.pasteur.fr/seqanal/protein/intro-uk.html

Antigenic: finds antigenic sites in proteins.

Helixturnhelix: reports nucleic acid binding motifs in your protein of interest.

Accessing Completed Genomes

TOPICS:

1. **GeneDB.**
2. **TigrDB.**
3. **Ensembl.**
4. **NCBI Genomic Biology.**

Accessing Genomic Sequences:

There is no one resource available on the web that allows you to access all the available genomes. In this course we will take a look at 3 sites for accessing most of the genomic information that is available out there. These sites often contain similar information and it may be possible to get most of the information you require from just one of these sites, however, to get the maximum amount of information it is often worth having a look at all 3 of these sites. In this course we will primarily concentrate on accessing the Trypanosome, human bovine genome data, however, any of the examples that we describe can easily be applied to any of the available species. Remember that most of the genomes are still in a draft state and are subject to change as more sequence becomes available.

GeneDB - http://www.genedb.org/

What is GeneDB?

Funded as part of the Wellcome Trust Functional Genomics Development Initiative, the GeneDB project is aiming to develop and maintain curated database resources for three organisms: *Schizosaccharomyces pombe*, which has been completely sequenced, and the kinetoplastid protozoa *Leishmania major* and *Trypanosoma brucei*, whose genome sequences have yet to be completed. The goals are to capture, store and manage data for integration with emerging functional genomics and proteomics projects and to provide an easy-to-use, user-friendly interface, including a variety of graphical displays. It is envisaged that the generic database structure will subsequently be adopted to integrate datasets for other organisms, both prokaryotic and eukaryotic, that have been sequenced by the Sanger Institute Pathogen Sequencing Unit.

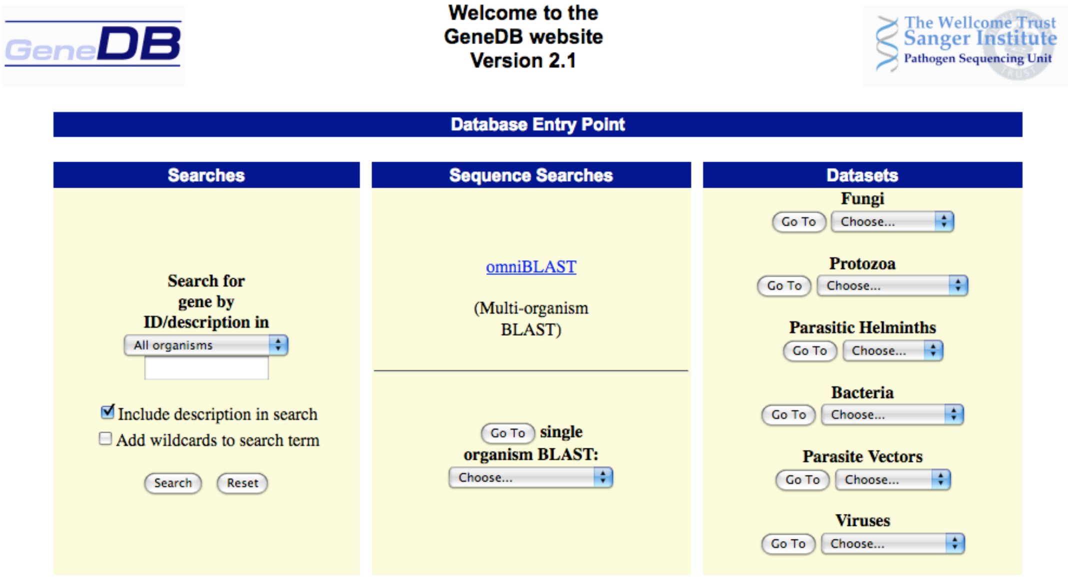
To this extend, datasets for *Saccharomyces cerevisiae* as well as the filamentous fungus *Aspergillus fumigatus* are already available through GeneDB. The database has been developed through close collaboration between Sanger Institute software developers, on-site organism specific curators and representatives of the research communities. The data within geneDB are manually annotated and curated, frequently updated and, because of the structured annotation and use of controlled vocabulary, easy to precisely query. The database is under constant review and new functionality will be added as it evolves.

What are the various ways to search GeneDB?

GeneDB provides users with the following information, functionality and research tools. The following are descriptions of ways to search GeneDB, where links will take you to the relevant areas of the database or to example pages. All the relevant search pages are available from a database entry point on both the GeneDB homepage and the individual organism homepages.

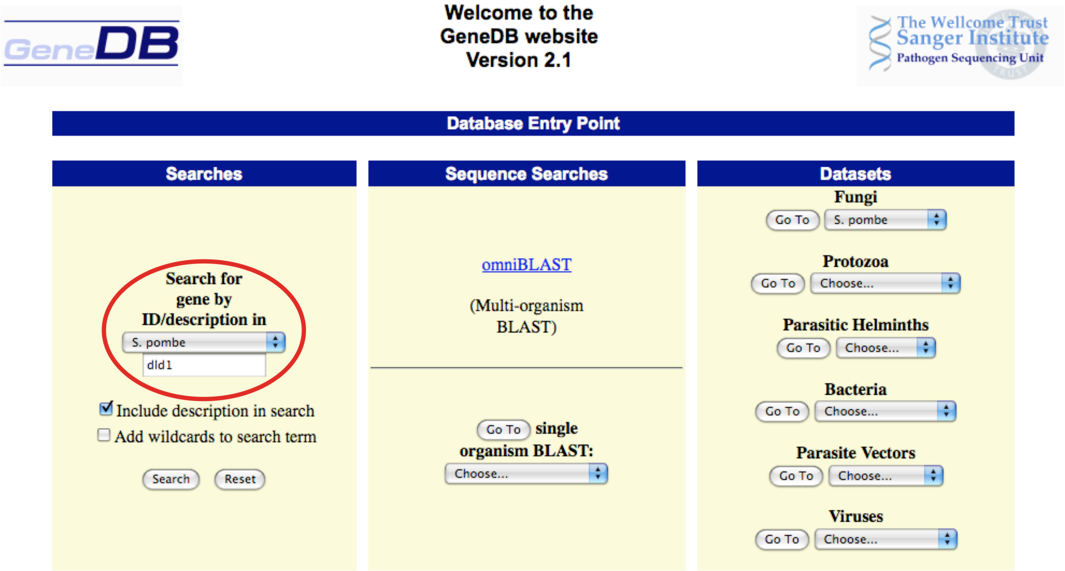
Menu Bar

To aid in the navigation of the site a menu bar is available on the pages within GeneDB. Most of the options available on GeneDB front pages are featured together with a comprehensive glossary of useful terms and databases. The menu bar has a gene search box, a drop down menu for the other organisms within GeneDB, Blast search and a link to the main search page. Also available are links to the GeneDB and organism front pages via the prominent GeneDB logos above the menu bar.


GeneDB
Welcome to the GeneDB website Version 2.1
The Wellcome Trust Sanger Institute Pathogen Sequencing Unit
Database Entry Point
Searches
Sequence Searches
Datasets
Search for gene by ID/description in
All organisms
Search
Reset
omniBLAST
(Multi-organism BLAST)
Go To
single organism BLAST:
Choose...
Fungi
Go To
Choose...
Protozoa
Go To
Choose...
Parasitic Helminths
Go To
Choose...
Bacteria
Go To
Choose...
Parasite Vectors
Go To
Choose...
Viruses
Go To
Choose...

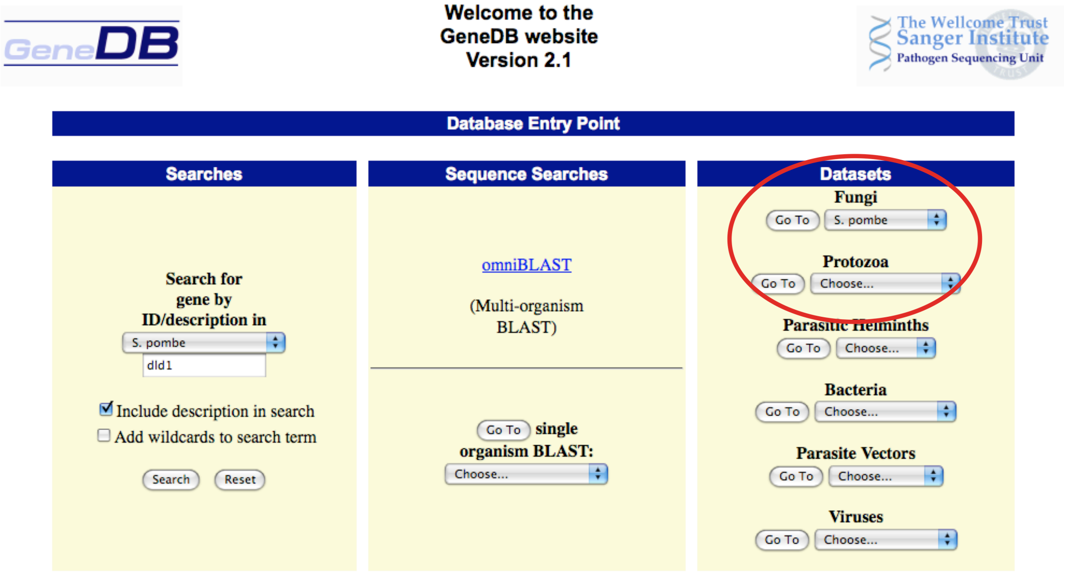
☑ Include description in search
☐ Add wildcards to search term

Searching for a gene by name or synonym

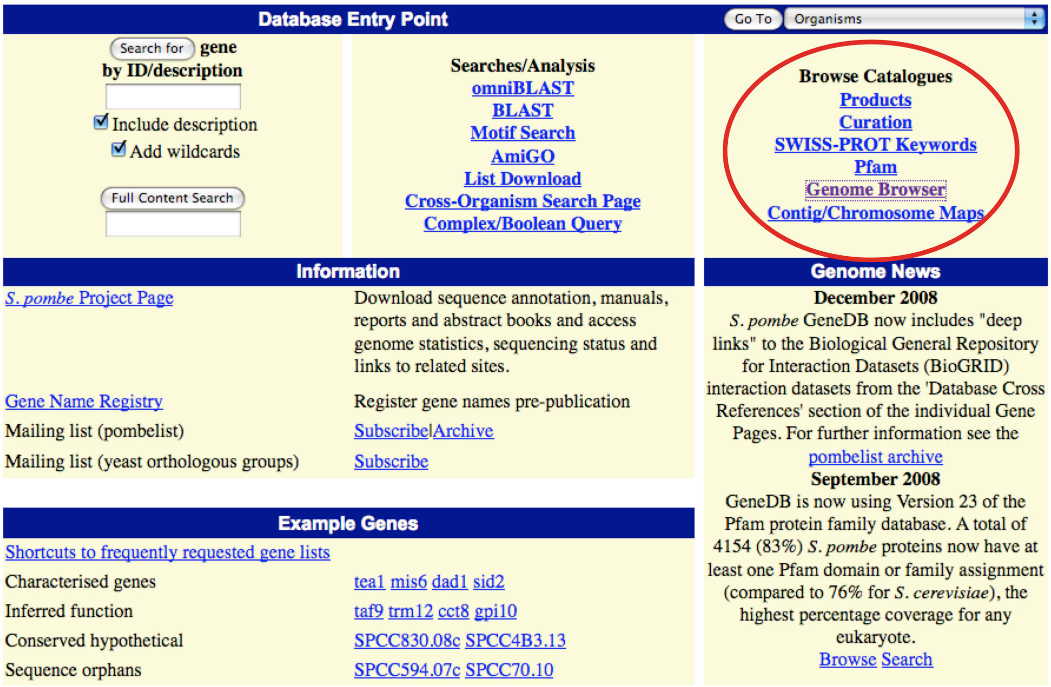

GeneDB
Welcome to the GeneDB website Version 2.1
The Wellcome Trust Sanger Institute Pathogen Sequencing Unit
Database Entry Point
Searches
Sequence Searches
Datasets
Search for gene by ID/description in
S. pombe
dld1
☑ Include description in search
☐ Add wildcards to search term
Search
Reset
omniBLAST
(Multi-organism BLAST)
Go To single organism BLAST:
Choose...
Fungi
Go To
S. pombe
Protozoa
Go To
Choose...
Parasitic Helminths
Go To
Choose...
Bacteria
Go To
Choose...
Parasite Vectors
Go To
Choose...
Viruses
Go To
Choose...

This option is also available on the menu bar of each gene page. Entering a gene name or synonym will lead either directly to the relevant gene page (eg dld1 in *S. pombe*) if a specific unique term is used or to a list of genes including that term (eg *kinase* in *T. brucei* and *L. major*) if a wild card is used. A list of genes will provide links to each relevant gene page.

Browsing by specific organism


GeneDB
Welcome to the GeneDB website Version 2.1
The Wellcome Trust Sanger Institute Pathogen Sequencing Unit
Database Entry Point
Searches
Sequence Searches
Datasets
Search for gene by ID/description in
S. pombe
dld1
☑ Include description in search
☐ Add wildcards to search term
Search
Reset
omniBLAST
(Multi-organism BLAST)
Go To single organism BLAST:
Choose...
Fungi
Go To
S. pombe
Protozoa
Go To
Choose...
Parasitic Helminths
Go To
Choose...
Bacteria
Go To
Choose...
Parasite Vectors
Go To
Choose...
Viruses
Go To
Choose...

Selecting a specific organism leads one to a page with brows able terms that includes:



Searches/Analysis
omniBLAST
BLAST
Motif Search
AmiGO
List Download
Cross-Organism Search Page
Complex/Boolean Query

Browse Catalogues
Products
Curation
SWISS-PROT Keywords
Pfam
Genome Browser
Contig/Chromosome Maps

Information

S. pombe Project Page	Download sequence annotation, manuals, reports and abstract books and access genome statistics, sequencing status and links to related sites.
Gene Name Registry	Register gene names pre-publication
Mailing list (pombelist)	Subscribe|Archive
Mailing list (yeast orthologous groups)	Subscribe

Example Genes

Shortcuts to frequently requested gene lists

Characterised genes	tea1 mis6 dad1 sid2
Inferred function	taf9 trm12 cct8 gpi10
Conserved hypothetical	SPCC830.08c SPCC4B3.13
Sequence orphans	SPCC594.07c SPCC70.10

Genome News

December 2008
S. pombe GeneDB now includes "deep links" to the Biological General Repository for Interaction Datasets (BioGRID) interaction datasets from the 'Database Cross References' section of the individual Gene Pages. For further information see the pombelist archive

September 2008
GeneDB is now using Version 23 of the Pfam protein family database. A total of 4154 (83%) *S. pombe* proteins now have at least one Pfam domain or family assignment (compared to 76% for *S. cerevisiae*), the highest percentage coverage for any eukaryote.
Browse Search

Products/description: This brings up a gene product list with links to relevant gene pages for each product

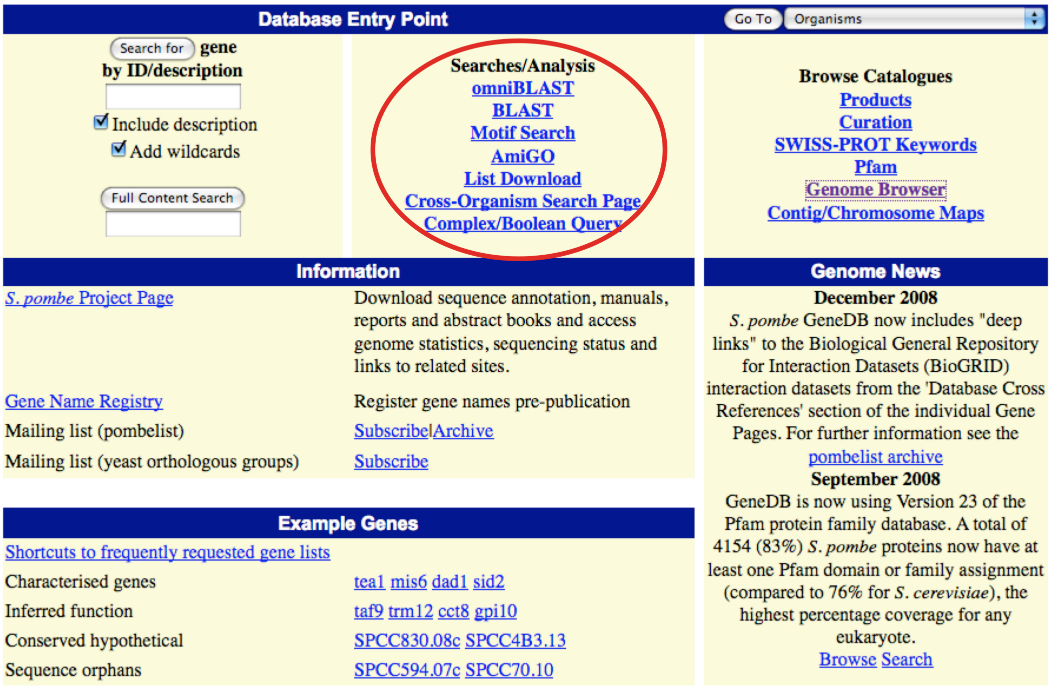
SWISS-PROT keywords: This is a browsable list of SWISS-PROT keywords assigned by SWISS-PROT curators to a given protein linking to the relevant gene or gene list pages

GO (Gene Ontology) list: This allows the user to search for genes by classification of their respective products into molecular function, biological process and cellular component using the controlled vocabulary defined by the GO consortium.

Pfam (domain data): This is a list of protein domain families defined by sequence alignments and hidden Markov models and a

Genome Browser: Visual inspection of genome and genes.

Sequence searching using BLAST



Database Entry Point

gene by ID/description

☑ Include description
☑ Add wildcards

Searches/Analysis
omniBLAST
BLAST
Motif Search
AmiGO
List Download
Cross-Organism Search Page
Complex/Boolean Query

Browse Catalogues
Products
Curation
SWISS-PROT Keywords
Pfam
Genome Browser
Contig/Chromosome Maps

Information

S. pombe Project Page	Download sequence annotation, manuals, reports and abstract books and access genome statistics, sequencing status and links to related sites.
Gene Name Registry	Register gene names pre-publication
Mailing list (pombelist)	Subscribe|Archive
Mailing list (yeast orthologous groups)	Subscribe

Example Genes

Shortcuts to frequently requested gene lists

Characterised genes	tea1 mis6 dad1 sid2
Inferred function	taf9 trm12 cct8 gpi10
Conserved hypothetical	SPCC830.08c SPCC4B3.13
Sequence orphans	SPCC594.07c SPCC70.10

Genome News

December 2008

S. pombe GeneDB now includes "deep links" to the Biological General Repository for Interaction Datasets (BioGRID) interaction datasets from the 'Database Cross References' section of the individual Gene Pages. For further information see the pombelist archive

September 2008

GeneDB is now using Version 23 of the Pfam protein family database. A total of 4154 (83%) *S. pombe* proteins now have at least one Pfam domain or family assignment (compared to 76% for *S. cerevisiae*), the highest percentage coverage for any eukaryote.

Browse Search

The BLAST and omniBLAST links lead to self-explanatory search pages, allowing users to paste in any nucleotide or amino acid sequence and compare it for similarity to any sequence within the database. Results are returned either directly or by e-mail.

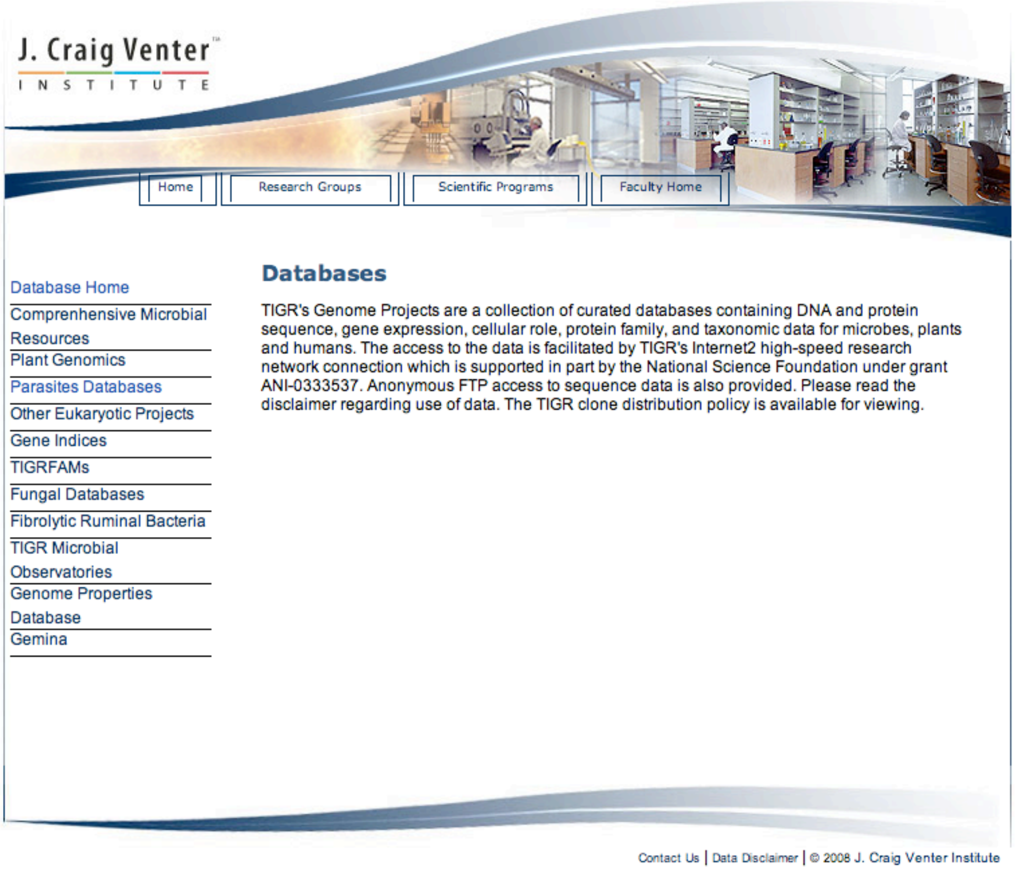
What kinds of information are in GeneDB?

Central to GeneDB are the gene pages, providing a comprehensive annotation of genes within each organism with:

3. Access DNA and protein sequences of protein coding genes with the ability of sending sequences straight to the associated BLAST servers
4. Predicted peptide properties (including signal peptide and transmembrane predictions)
5. Similarity information (EMBL, SWISS-PROT including annotation)
6. Gene ontology (GO) annotation
7. Summary of up-to-date protein domain and motif searches (InterPro, Pfam, PRINTS, PROSITE, BLOCKS, SMART)
8. Literature links
9. SWISS-PROT annotation

TigrDB - http://www.tigr.org/db.shtml

TIGR's Genome Projects are a collection of curated databases containing DNA and protein sequence, gene expression, cellular role, protein family, and taxonomic data for microbes, plants and humans. Anonymous FTP access to sequence data is also provided. Please read the disclaimer regarding use of data. The TIGR clone distribution policy is available for viewing. There are several databases available on the TIGR database website: http://www.tigr.org/db.shtml. Several types of databases are found on the TIGR website. One is for completed and unfinished parasite genome sequences, sequenced at TIGR. A second valuable type of databases are the TIGR Gene Index.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Plant Genomics
Parasites Databases
Other Eukaryotic Projects
Gene Indices
TIGRFAMs
Fungal Databases
Fibrolytic Ruminal Bacteria
TIGR Microbial Observatories
Genome Properties Database
Gemina

Databases

TIGR's Genome Projects are a collection of curated databases containing DNA and protein sequence, gene expression, cellular role, protein family, and taxonomic data for microbes, plants and humans. The access to the data is facilitated by TIGR's Internet2 high-speed research network connection which is supported in part by the National Science Foundation under grant ANI-0333537. Anonymous FTP access to sequence data is also provided. Please read the disclaimer regarding use of data. The TIGR clone distribution policy is available for viewing.

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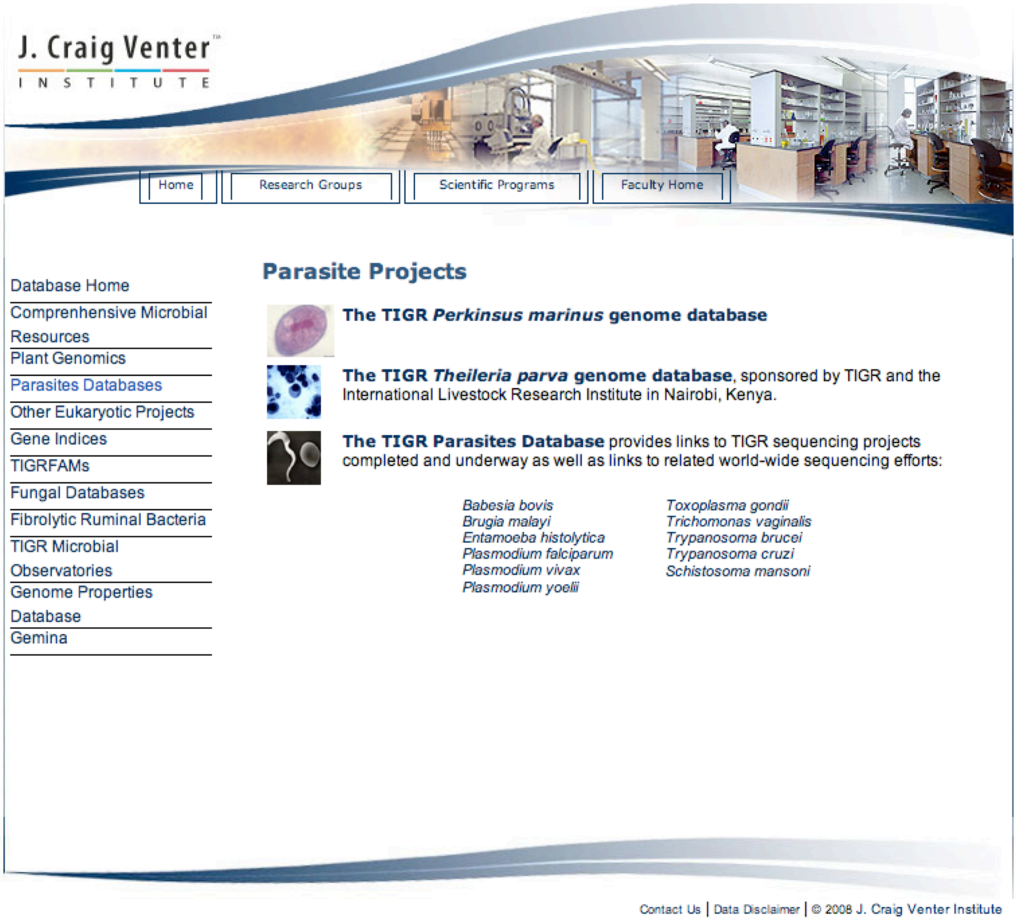
Parasites Databases

Under the Parasites Databases, several completed parasite databases are found including data on several uncompleted genomes i.e. *Trypanosoma brucei*.


J. Craig Venter™
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Parasite Projects

The TIGR *Perkinsus marinus* genome database

The TIGR *Theileria parva* genome database, sponsored by TIGR and the International Livestock Research Institute in Nairobi, Kenya.

The TIGR Parasites Database provides links to TIGR sequencing projects completed and underway as well as links to related world-wide sequencing efforts:

Babesia bovis
Brugia malayi
Entamoeba histolytica
Plasmodium falciparum
Plasmodium vivax
Plasmodium yoelii
Toxoplasma gondii
Trichomonas vaginalis
Trypanosoma brucei
Trypanosoma cruzi
Schistosoma mansoni

Data in the database can be accessed through several methods:

Gene Name Search Text search of the putative identifications in the Gene Identification Table.

Locus Search Obtain a report on a predicted coding region by locus number.

Sequence Search Provides searching of nucleotide or peptide sequences against predicted coding regions or the chromosomes.

HMM Search Search a sequence against protein family based HMMs

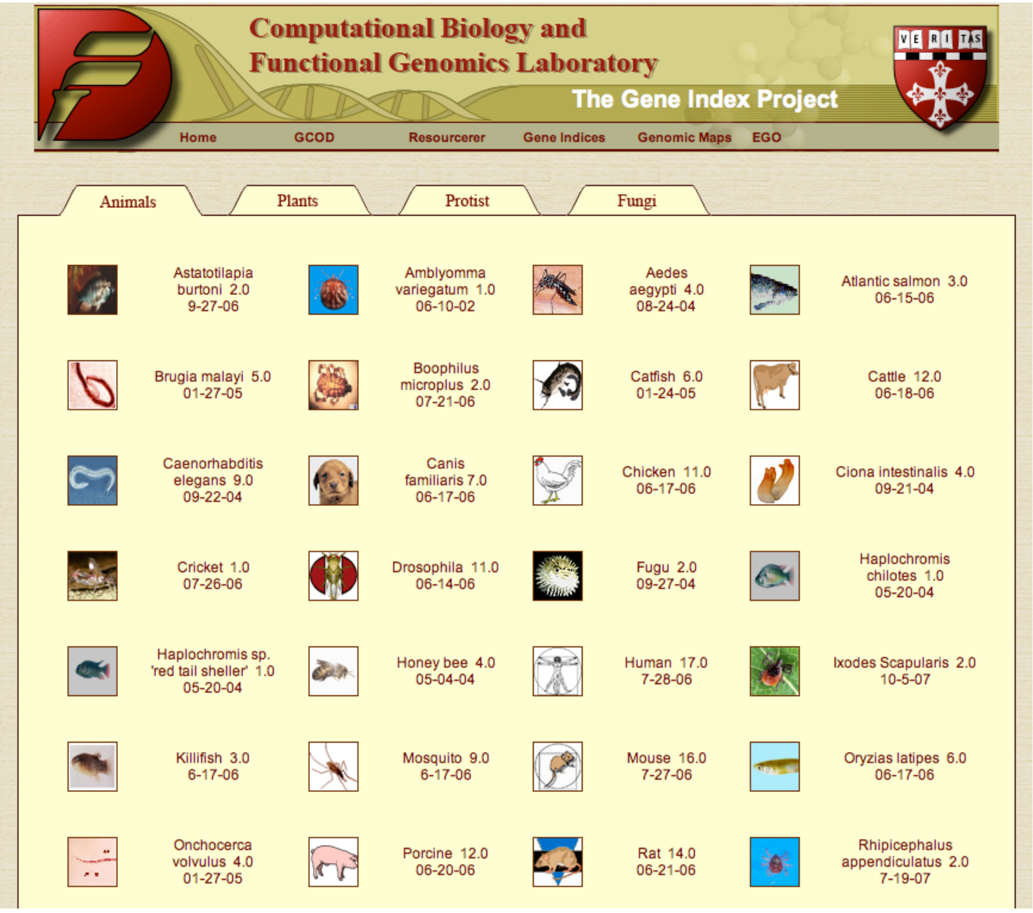
View Chromosomes Browse the chromosomes or retrieve a table of clones sorted by chromosome.

Gene Index project - http://compbio.dfci.harvard.edu/tgi/

The promise of genome projects has been a complete catalogue of genes in a wide range of organisms. While genome projects have been successful in providing reference genome sequences, the problem of finding genes and their variants in genomic sequence remains an ongoing challenge.

The sequencing of Expressed Sequence Transcripts (ESTs), fragments of genes that have been copied from DNA to RNA, provides the most comprehensive evidence for the existence of genes and their structure.

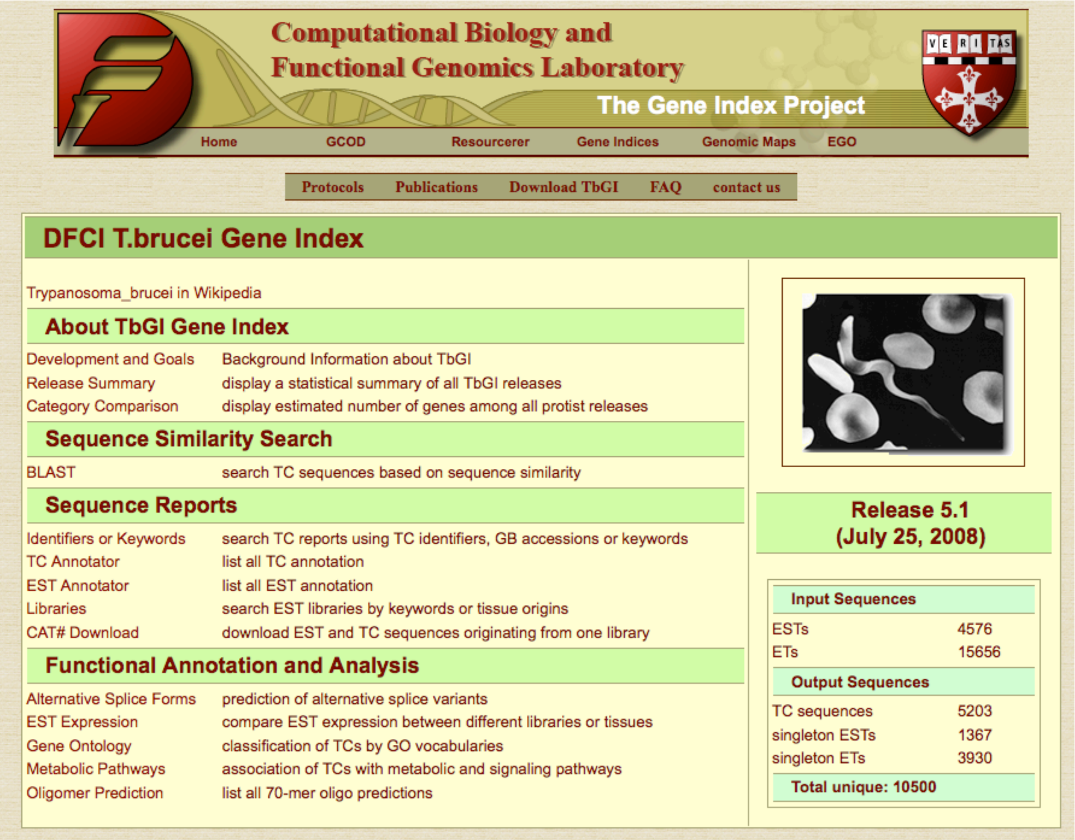
The goal of The Gene Index Project is to use the available EST and gene sequences, along with the reference genomes wherever available, to provide an inventory of likely genes and their variants and to annotate these with information regarding the functional roles played by these genes and their products. In addition, they are attempting to use these catalogues to find links between genes and pathways in different species and to provide lists of features within completed genomes that can aid in the understanding of how gene expression is regulated.



Astatotilapia burtoni 2.0 9-27-06	Amblyomma variegatum 1.0 06-10-02	Aedes aegypti 4.0 08-24-04	Atlantic salmon 3.0 06-15-06
Brugia malayi 5.0 01-27-05	Boophilus microplus 2.0 07-21-06	Catfish 6.0 01-24-05	Cattle 12.0 06-18-06
Caenorhabditis elegans 9.0 09-22-04	Canis familiaris 7.0 06-17-06	Chicken 11.0 06-17-06	Ciona intestinalis 4.0 09-21-04
Cricket 1.0 07-26-06	Drosophila 11.0 06-14-06	Fugu 2.0 09-27-04	Haplochromis chilotes 1.0 05-20-04
Haplochromis sp. 'red tail sheller' 1.0 05-20-04	Honey bee 4.0 05-04-04	Human 17.0 7-28-06	Ixodes Scapularis 2.0 10-5-07
Killifish 3.0 6-17-06	Mosquito 9.0 6-17-06	Mouse 16.0 7-27-06	Oryzias latipes 6.0 06-17-06
Onchocerca volvulus 4.0 01-27-05	Porcine 12.0 06-20-06	Rat 14.0 06-21-06	Rhipicephalus appendiculatus 2.0 7-19-07

Example *T.brucei* Gene Index - http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=t_brucei

The DFCI *T. brucei* Gene Index integrates research data from international *T. brucei* EST sequencing and gene research projects. The ultimate goal of the DFCI Gene Index projects, including TbGI, is to represent a non-redundant view of all *T. brucei* genes and data on their expression patterns, cellular roles, functions, and evolutionary relationships.



DFCI T.brucei Gene Index

Trypanosoma_brucei in Wikipedia

About TbGI Gene Index

Development and Goals	Background Information about TbGI
Release Summary	display a statistical summary of all TbGI releases
Category Comparison	display estimated number of genes among all protist releases

Sequence Similarity Search

BLAST	search TC sequences based on sequence similarity

Sequence Reports

Identifiers or Keywords	search TC reports using TC identifiers, GB accessions or keywords
TC Annotator	list all TC annotation
EST Annotator	list all EST annotation
Libraries	search EST libraries by keywords or tissue origins
CAT# Download	download EST and TC sequences originating from one library

Functional Annotation and Analysis

Alternative Splice Forms	prediction of alternative splice variants
EST Expression	compare EST expression between different libraries or tissues
Gene Ontology	classification of TCs by GO vocabularies
Metabolic Pathways	association of TCs with metabolic and signaling pathways
Oligomer Prediction	list all 70-mer oligo predictions

Release 5.1
(July 25, 2008)

Input Sequences	
ESTs	4576
ETs	15656
Output Sequences	
TC sequences	5203
singleton ESTs	1367
singleton ETs	3930
Total unique: 10500	

Data can be accessed through several means:

[BLAST]() search TC sequences based on sequence similarity

[Identifiers or Keywords]() search TC reports using TC identifiers, GB accessions or keywords

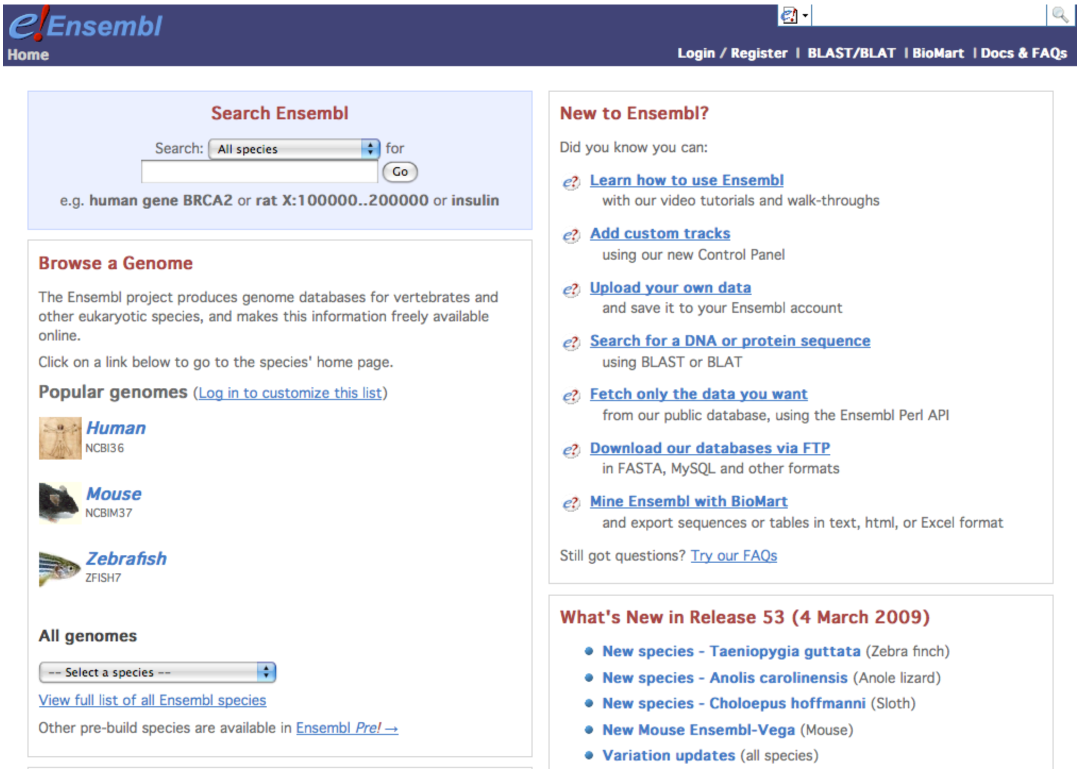
[TC Annotator]() list all TC annotation

[EST Annotator]() list all EST annotation

And several other.

ENSEMBL - http://www.ensembl.org/

Ensembl is a joint project between EMBL - EBI and the Sanger Institute to develop a software system which produces and maintains automatic annotation on eukaryotic genomes. A wide range of genomes is available.



Search Ensembl

e.g. **human gene BRCA2** or **rat X:100000..200000** or **insulin**

Browse a Genome

The Ensembl project produces genome databases for vertebrates and other eukaryotic species, and makes this information freely available online.

Click on a link below to go to the species' home page.

Popular genomes (Log in to customize this list)

Human NCBI36

Mouse NCBIM37

Zebrafish ZFISH7

All genomes

View full list of all Ensembl species

Other pre-build species are available in Ensembl *Pre!* →

New to Ensembl?

Did you know you can:

- Learn how to use Ensembl with our video tutorials and walk-throughs
- Add custom tracks using our new Control Panel
- Upload your own data and save it to your Ensembl account
- Search for a DNA or protein sequence using BLAST or BLAT
- Fetch only the data you want from our public database, using the Ensembl Perl API
- Download our databases via FTP in FASTA, MySQL and other formats
- Mine Ensembl with BioMart and export sequences or tables in text, html, or Excel format

Still got questions? Try our FAQs

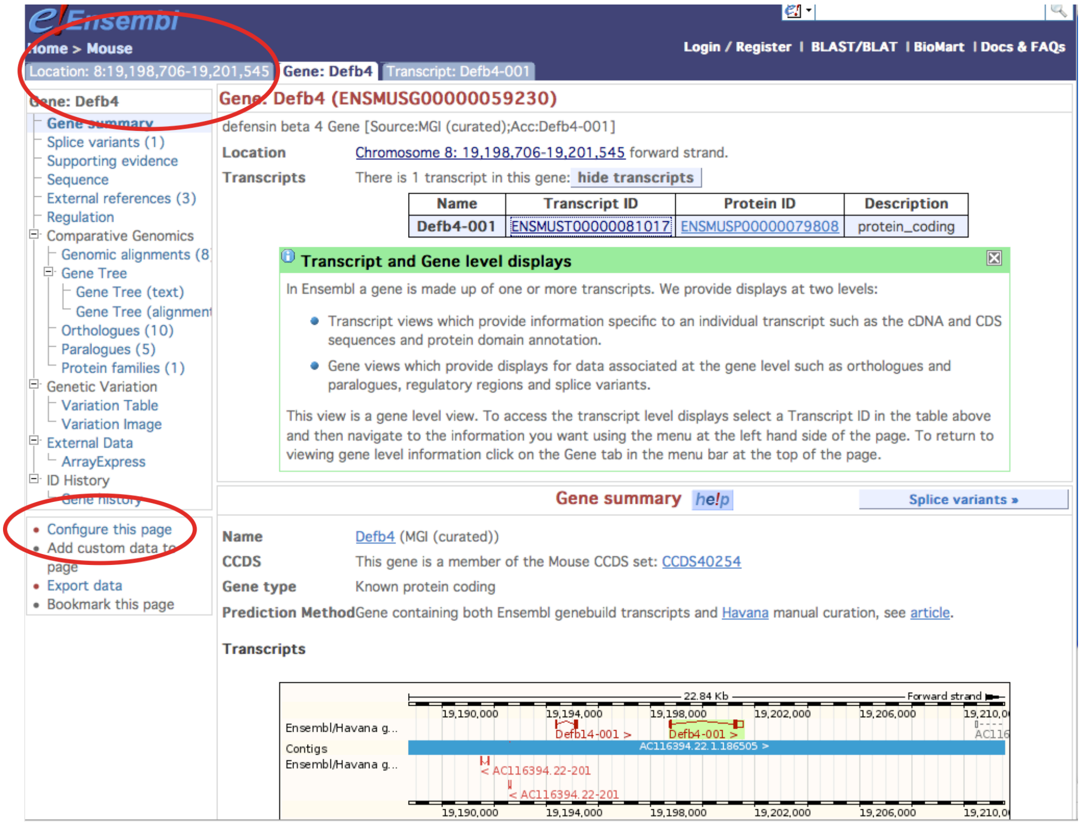
What's New in Release 53 (4 March 2009)

- **New species - Taeniopygia guttata** (Zebra finch)
- **New species - Anolis carolinensis** (Anole lizard)
- **New species - Choloepus hoffmanni** (Sloth)
- **New Mouse Ensembl-Vega** (Mouse)
- **Variation updates** (all species)

- Click on one of the species to access the genomic information e.g. Mouse.
- To find your gene of interest you can enter in the empty box (top right-hand corner of page), the gene symbol, gene accession number, mRNA accession number, SwissProt accession number, EST accession number etc.
- You can also access the genome by chromosome number.
- There are a number of useful links located on the right side of the page for new users.
  - Learn how to use Ensembl.
  - Upload you own data.
  - Search Ensembl using Blast or Blat.
  - Data mine Ensembl with BioMart.
  - Download data – ftp download of data (mostly used for large bioinformatics projects).
  -

Example: Mouse beta-defensin 4 (defB4).

- Select Mouse from the pull-down menu in the “Search Ensembl” box and type the gene RefSeq symbol in the empty box (defB4).
- Click “Go”.
- This will take you to a query results page. In this case there are 3 entries.
- Click on the **Ensembl protein_coding Gene:** ENSMUSG00000059230 link for information on your gene such as its sequence, structure, domains that it contains etc.
- A gene summary page will be displayed and a graphical display of the gene in the genome similar to the UCSC browser.


Ensembl
Home > Mouse
Login / Register | BLAST/BLAT | BioMart | Docs & FAQs
Location: 8:19,198,706-19,201,545
Gene: Defb4
Transcript: Defb4-001
Gene: Defb4
Gene summary
Splice variants (1)
Supporting evidence
Sequence
External references (3)
Regulation
Comparative Genomics
Genomic alignments (8
Gene Tree
Gene Tree (text)
Gene Tree (alignment
Orthologues (10)
Paralogues (5)
Protein families (1)
Genetic Variation
Variation Table
Variation Image
External Data
ArrayExpress
ID History
Gene history
Configure this page
Add custom data to page
Export data
Bookmark this page
hide transcripts
Gene summary
help
Splice variants »
22.84 Kb
Forward strand
19,190,000
19,194,000
19,198,000
19,202,000
19,206,000
19,210,0
Ensembl/Havana g...
Defb14-001 >
Defb4-001 >
AC116
Contigs
AC116394.22.1.186505 >
Ensembl/Havana g...
< AC116394.22-201
< AC116394.22-201

Gene: Defb4 (ENSMUSG00000059230)

defensin beta 4 Gene [Source:MGI (curated);Acc:Defb4-001]

Location Chromosome 8: 19,198,706-19,201,545 forward strand.

Transcripts There is 1 transcript in this gene:

Name	Transcript ID	Protein ID	Description
Defb4-001	ENSMUST00000081017	ENSMUSP00000079808	protein_coding

Transcript and Gene level displays

In Ensembl a gene is made up of one or more transcripts. We provide displays at two levels:

- Transcript views which provide information specific to an individual transcript such as the cDNA and CDS sequences and protein domain annotation.
- Gene views which provide displays for data associated at the gene level such as orthologues and paralogues, regulatory regions and splice variants.

This view is a gene level view. To access the transcript level displays select a Transcript ID in the table above and then navigate to the information you want using the menu at the left hand side of the page. To return to viewing gene level information click on the Gene tab in the menu bar at the top of the page.

Name Defb4 (MGI (curated))

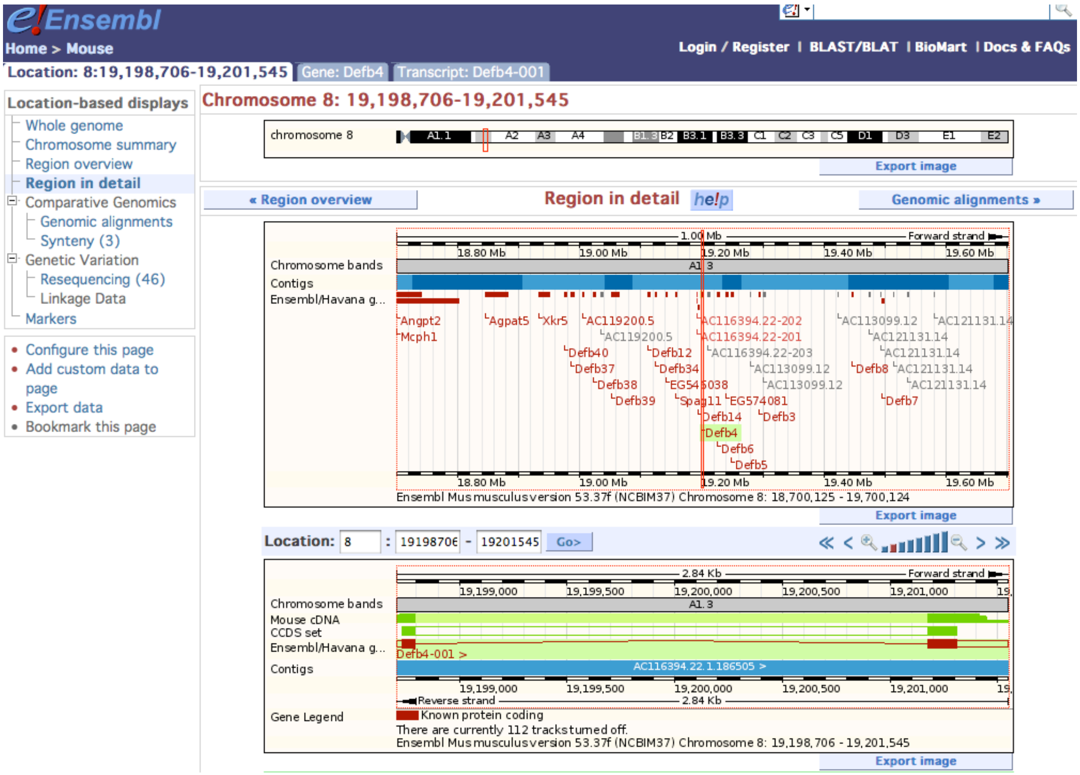
CCDS This gene is a member of the Mouse CCDS set: CCDS40254

Gene type Known protein coding

Prediction Method Gene containing both Ensembl genebuild transcripts and Havana manual curation, see article.

Transcripts

Additional data in this region can be viewed by using the "Configure this page" link on the left. Selecting the “Location” tab on the top Left, gives an overview of the location of gene in the genome.

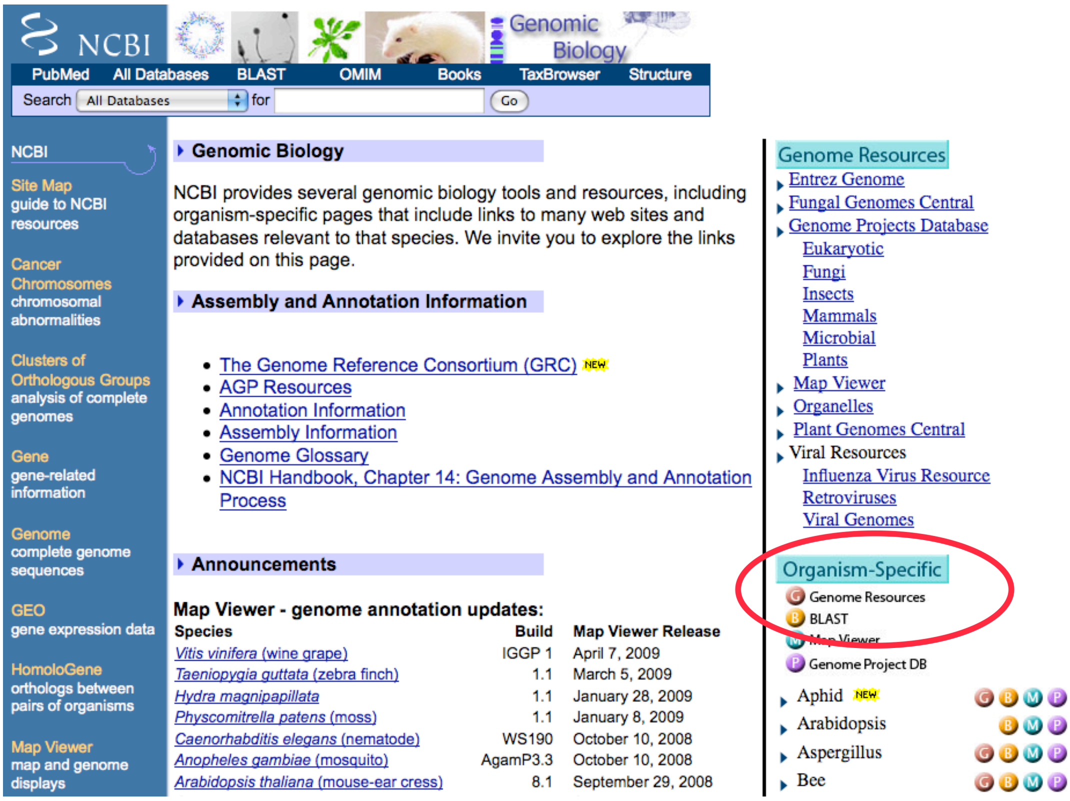

Ensembl
Home > Mouse
Login / Register | BLAST/BLAT | BioMart | Docs & FAQs
Location: 8:19,198,706-19,201,545
Gene: Defb4
Transcript: Defb4-001
Location-based displays
Whole genome
Chromosome summary
Region overview
Region in detail
Comparative Genomics
Genomic alignments
Synteny (3)
Genetic Variation
Resequencing (46)
Linkage Data
Markers
Configure this page
Add custom data to page
Export data
Bookmark this page
Chromosome 8: 19,198,706-19,201,545
chromosome 8
Export image
« Region overview
Region in detail
help
Genomic alignments »
Chromosome bands
Contigs
Ensembl/Havana g...
Angpt2
Mcph1
Agpat5
Xkr5
Defb40
Defb37
Defb38
Defb39
Defb12
Defb34
Defb14
Defb4
Defb6
Defb5
Defb3
Defb8
Defb7
Ensembl Mus musculus version 53.37f (NCBIM37) Chromosome 8: 18,700,125 - 19,700,124
Location: 8 : 19198706 - 19201545 Go>
Chromosome bands
Mouse cDNA
CCDS set
Ensembl/Havana g...
Defb4-001 >
Contigs
AC116394.22.1.186505 >
Forward strand
Reverse strand
2.84 Kb
Gene Legend
Known protein coding
There are currently 112 tracks turned off.
Ensembl Mus musculus version 53.37f (NCBIM37) Chromosome 8: 19,198,706 - 19,201,545

In this view only 7 tracks are shown in the overview panel and 112 tracks in the main panel turned off. To change the tracks you are displaying, use the "Configure this page" link on the left.

NCBI - http://www.ncbi.nlm.nih.gov/Genomes/index.html

Many of you will be familiar with the National Center for Biotechnology Information (NCBI) website which has many very useful resources including; Entrez, PubMed, Genbank, BLAST, OMIM. Today we will see how to use the NCBI site to interrogate the genomic sequences that are available there.

The NCBI site provides a good starting point for accessing the widest range of eukaryotic and microbial genomes. Many of these genomes will have their own dedicated sites located at other websites, but the NCBI site will provide links to them.


NCBI
Genomic Biology
PubMed
All Databases
BLAST
OMIM
Books
TaxBrowser
Structure
Search
All Databases
for
Go
NCBI
Site Map
guide to NCBI resources
Cancer Chromosomes
chromosomal abnormalities
Clusters of Orthologous Groups
analysis of complete genomes
Gene
gene-related information
Genome
complete genome sequences
GEO
gene expression data
HomoloGene
orthologs between pairs of organisms
Map Viewer
map and genome displays
Genome Resources
Entrez Genome
Fungal Genomes Central
Genome Projects Database
Eukaryotic
Fungi
Insects
Mammals
Microbial
Plants
Map Viewer
Organelles
Plant Genomes Central
Viral Resources
Influenza Virus Resource
Retroviruses
Viral Genomes
Organism-Specific
G Genome Resources
B BLAST
M Map Viewer
P Genome Project DB
Aphid NEW G B M P
Arabidopsis B M P
Aspergillus G B M P
Bee G B M P

Genomic Biology

NCBI provides several genomic biology tools and resources, including organism-specific pages that include links to many web sites and databases relevant to that species. We invite you to explore the links provided on this page.

Assembly and Annotation Information

- The Genome Reference Consortium (GRC) NEW
- AGP Resources
- Annotation Information
- Assembly Information
- Genome Glossary
- NCBI Handbook, Chapter 14: Genome Assembly and Annotation Process

Announcements

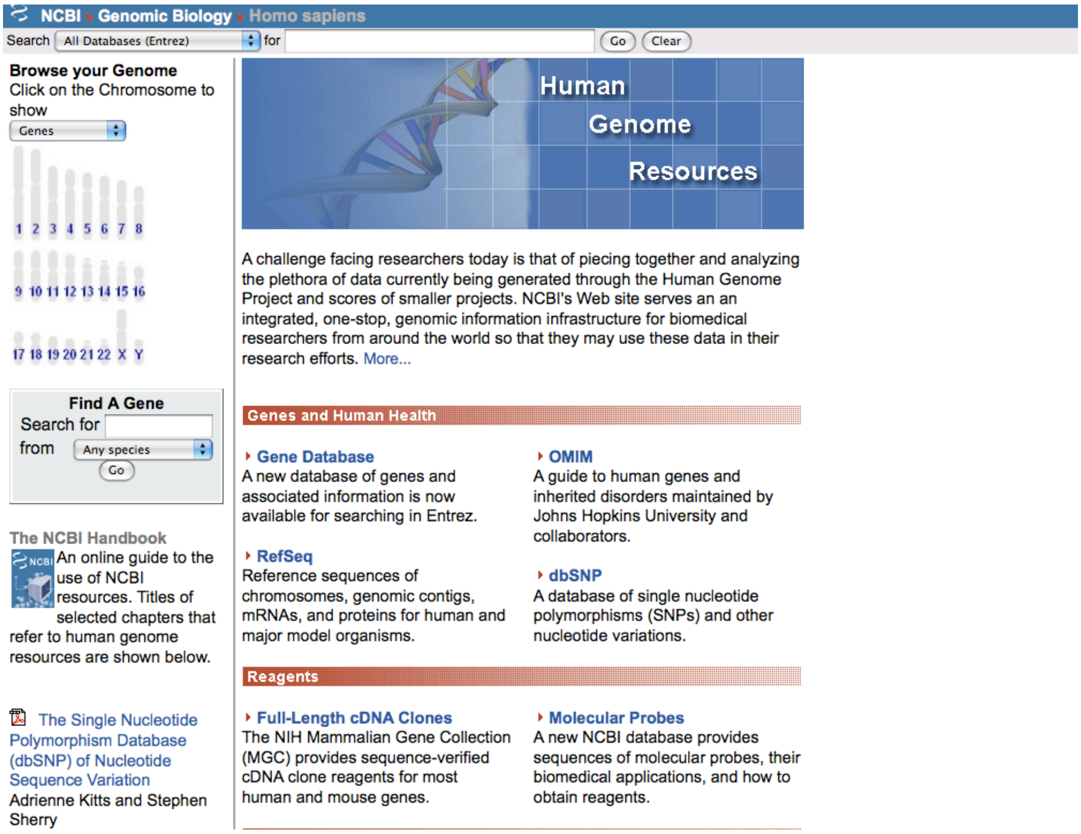
Map Viewer - genome annotation updates:

Species	Build	Map Viewer Release
Vitis vinifera (wine grape)	IGGP 1	April 7, 2009
Taeniopygia guttata (zebra finch)	1.1	March 5, 2009
Hydra magnipapillata	1.1	January 28, 2009
Physcomitrella patens (moss)	1.1	January 8, 2009
Caenorhabditis elegans (nematode)	WS190	October 10, 2008
Anopheles gambiae (mosquito)	AgamP3.3	October 10, 2008
Arabidopsis thaliana (mouse-ear cress)	8.1	September 29, 2008

Accessing the Human Genome:

To access the human genome - go to the URL above and click on the Human genome Resources button under the Organism-Specific column on the left. This page provides a number of links such as a link to BLAST where you can search your sequence against the human genome. You can also browse the genome by chromosome by clicking on one of the chromosomes.

The best way to access the genome if you have a particular gene of interest is to search for your gene in Entrez Gene. Entrez Gene provides a single query interface to curated sequence and descriptive information about genetic loci. It presents information on official nomenclature, aliases, sequence accessions, phenotypes, EC numbers, MIM numbers, UniGene clusters, homology, map locations, and related web sites.



Browse your Genome
Click on the Chromosome to show

Find A Gene

The NCBI Handbook
An online guide to the use of NCBI resources. Titles of selected chapters that refer to human genome resources are shown below.

The Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation
Adrienne Kitts and Stephen Sherry

A challenge facing researchers today is that of piecing together and analyzing the plethora of data currently being generated through the Human Genome Project and scores of smaller projects. NCBI's Web site serves an an integrated, one-stop, genomic information infrastructure for biomedical researchers from around the world so that they may use these data in their research efforts. More...

Genes and Human Health

Gene Database
A new database of genes and associated information is now available for searching in Entrez.

OMIM
A guide to human genes and inherited disorders maintained by Johns Hopkins University and collaborators.

RefSeq
Reference sequences of chromosomes, genomic contigs, mRNAs, and proteins for human and major model organisms.

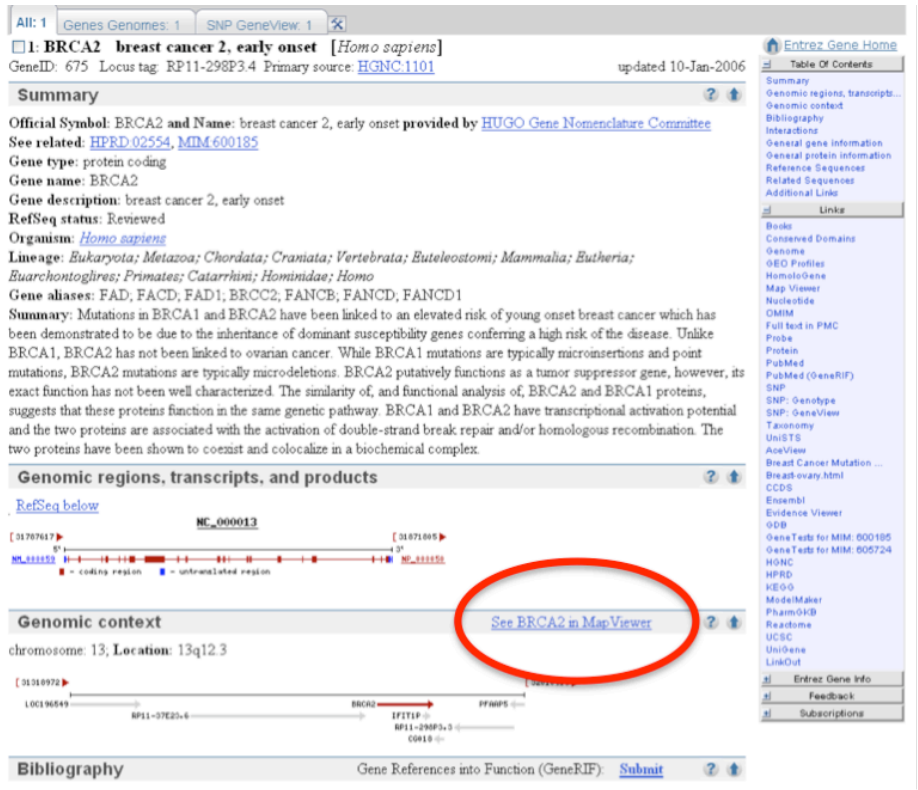
dbSNP
A database of single nucleotide polymorphisms (SNPs) and other nucleotide variations.

Reagents

Full-Length cDNA Clones
The NIH Mammalian Gene Collection (MGC) provides sequence-verified cDNA clone reagents for most human and mouse genes.

Molecular Probes
A new NCBI database provides sequences of molecular probes, their biomedical applications, and how to obtain reagents.

- Follow the "Gene Database" link on the Human Genome Resources page.
- At the top of the page search Entrez Gene by entering your gene name (full name, abbreviation or accession number) in the box and "Go". (Example: BRCA2)
- This brings up a results page that matches the query for some reason.
- You can use the limits section to limit your search by various criteria such as organism.
- Click on BRCA2 (i.e. **GeneID** 675) to take you to the Entrez Gene page for that gene.


All: 1
Genes Genomes: 1
SNP GeneView: 1
Entrez Gene Home
Table Of Contents
Summary
Genomic regions, transcripts...
Genomic context
Bibliography
Interactions
General gene information
General protein information
Reference Sequences
Related Sequences
Additional Links
Links
Books
Conserved Domains
Genome
GEO Profiles
HomoloGene
Map Viewer
Nucleotide
OMIM
Full text in PMC
Probe
Protein
PubMed
PubMed (GeneRIF)
SNP
SNP: Genotype
SNP: GeneView
Taxonomy
UniSTS
AceView
Breast Cancer Mutation ...
Breast-ovary.html
CCDS
Ensembl
Evidence Viewer
GDB
GeneTests for MIM: 600185
GeneTests for MIM: 605724
HGNC
HPRD
KEGG
ModelMaker
PharmGKB
Reactome
UCSC
UniGene
LinkOut
Entrez Gene Info
Feedback
Subscriptions
RefSeq below
NC_000013
See BRCA2 in MapViewer
Submit

☐1: **BRCA2 breast cancer 2, early onset** [*Homo sapiens*]

GeneID: 675 Locus tag: RP11-298P3.4 Primary source: HGNC:1101 updated 10-Jan-2006

Summary

Official Symbol: BRCA2 **and Name**: breast cancer 2, early onset **provided by** HUGO Gene Nomenclature Committee

See related: HPRD:02554, MIM:600185

Gene type: protein coding

Gene name: BRCA2

Gene description: breast cancer 2, early onset

RefSeq status: Reviewed

Organism: *Homo sapiens*

Lineage: *Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini; Hominidae; Homo*

Gene aliases: FAD; FACD; FAD1; BRCC2; FANCB; FANCD; FANCD1

Summary: Mutations in BRCA1 and BRCA2 have been linked to an elevated risk of young onset breast cancer which has been demonstrated to be due to the inheritance of dominant susceptibility genes conferring a high risk of the disease. Unlike BRCA1, BRCA2 has not been linked to ovarian cancer. While BRCA1 mutations are typically microinsertions and point mutations, BRCA2 mutations are typically microdeletions. BRCA2 putatively functions as a tumor suppressor gene, however, its exact function has not been well characterized. The similarity of, and functional analysis of, BRCA2 and BRCA1 proteins, suggests that these proteins function in the same genetic pathway. BRCA1 and BRCA2 have transcriptional activation potential and the two proteins are associated with the activation of double-strand break repair and/or homologous recombination. The two proteins have been shown to coexist and colocalize in a biochemical complex.

Genomic regions, transcripts, and products

Genomic context

chromosome: 13; **Location**: 13q12.3

Bibliography

Gene References into Function (GeneRIF): Submit

Starting at the top of the page:

- A graphic of the BRCA2 transcript is shown including the intron-exon structure. You can click on this graphic to obtain the sequence.
- This is followed by a graphic showing BRCA2 in its genomic context i.e. what genes are located around it.
- This is followed by various information on the gene including;

Gene aliases – other names for the gene.

Summary - written by staff of the NCBI RefSeq group describing the function, localization, and sequence properties of the gene and its products.

Bibliography – a detailed list of PubMed entries for the gene.

Interactions – What other genes/proteins are known to interact with BRCA2.

A General Gene Information Section – includes the official gene symbol and name, gene ontology details, homology with mouse and rat, etc.

There is also a link to the NCBI Map Viewer (see below).

NCBI Reference Sequences (RefSeq) - All RefSeq records created for a given locus are listed. Multiple records are distinguished by the brief description of the transcript variant. This section provides links to:

RefSeq nucleotide record (genomic and mRNA accessions have 'NG_' and 'NM_' prefixes, respectively).

RefSeq Product - protein record (the 'NP_' prefix).

Conserved domains found in the protein.

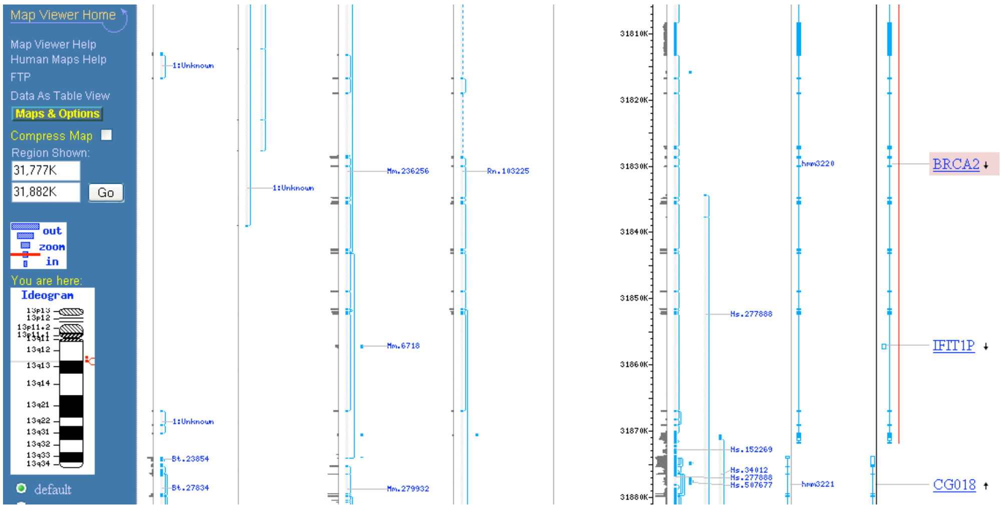
Related Sequences - A table of a subset of representative nucleotide and protein accessions for the locus. EST accession numbers are provided if no other sequence data are available to represent the locus.

Additional Links - This section names and provides links to additional sites that may contain information related to this locus such as OMIM, UniGene, etc.

MAP VIEWER:

This is the NCBI graphical display tool, which you can use to display the genomic context of your sequence. This tool is not as user friendly or as advanced as the UCSC or Ensembl browsers and we recommend that you use these to view the genome graphically where possible. Not all species are available at these sites so you may need to use Map viewer.

- Click on "Maps & Options" to choose which features you wish to display.
- Click on any of the genes, RNAs or Unigenes to get more information.
- You can download genomic sequence for the region selected using the "Download/View Sequence/Evidence" link.



Compress Map ☐

Region Shown:
31,777K
31,882K

● default

Map Viewer Home
Map Viewer Help
Human Maps Help
FTP
Data As Table View
Maps & Options
Go
out
zoom
in
You are here:
Ideogram
13p13
13p12
13p11.2
13p11.1
13q11
13q12
13q13
13q14
13q21
13q22
13q31
13q32
13q33
13q34
1:Unknown
1:Unknown
1:Unknown
Bt.23854
Bt.27834
Mm.236256
Mm.6718
Mm.279932
Rn.103225
31810K
31820K
31830K
31840K
31850K
31860K
31870K
31880K
Hs.277888
Hs.152269
Hs.34012
Hs.277888
Hs.507677
hmm3220
hmm3221
BRCA2
IFIT1P
CG018

Accessing the Other Genomes: http://www.ncbi.nlm.nih.gov/Genomes/index.html

Plant Genomes Central

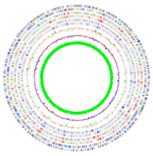
The plant genomic effort has one technical hurdle relative to other genomic efforts. The range of plant genome size is very large; extending from approximately the same size as small animals to more than five times as large as human. At NCBI resources for many plant species are available including;

- *Arabidopsis thaliana* (thale cress)
- *Gossypium* (cotton)
- *Hordeum vulgare* (barley)
- *Lycopersicon esculentum* (tomato)
- *Medicago truncatula* (barrel medic)
- *Oryza sativa* (rice)
- *Solanum tuberosum* (potato)
- *Triticum aestivum* (bread wheat)
- *Zea mays* (corn)

Malaria Parasite

This resource provides data and information relevant to malaria genetics and genomics following the sequencing of the malaria parasite *Plasmodium falciparum* and one of its major vectors Anopheles gambiae genomes. These resources include;

- Organism specific sequence BLAST databases.
- Genome maps & linkage markers.
- Information about genetic studies.
- Links to other malaria web sites.
    - Genetic data on related apicomplexan parasites.



Microbial Genomes

This resource provides links to the 279 (as of 07/11/2005) completely sequenced bacterial genomes (24 Archaea & 255 eubacteria). You can download information on the genome in a number of different formats

[T] - All proteins of the complete genome were searched against "nr" database. The detected homologs were classified into three taxonomic groups, Eukaryota, Eubacteria and Archaea in TaxTable.

[P] – Download the protein sequences from ProtTable.

[C] – Functional classifications are located in COG Table.

[D] - 3-D neighbors (proteins with sequence similarity to proteins with known 3D structure)

[L] – BLAST a sequence against the genome.

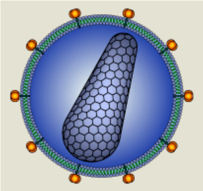
[S] - CDD search (list of conserved domains in proteins).

[F] – FTP data.

[R] – PubMed references.

For most of the genomes you can follow links to an organism-specific website with even further details (usually hosted by the sequencing consortium)

Retroviruses



Collection of resources at NCBI specifically designed to support the research of retroviruses. The resources include:

- Taxa-specific pages for HIV-1, HIV-2, SIV, HTLV, STLV.
- Genotyping tool - uses the BLAST algorithm to identify the genotype of a query sequence
- Alignment tool - global alignment of multiple sequences
- HIV-1 automatic sequence annotation - generates a report in GenBank format for one or more query sequences
- Genome maps - graphical representation of 50 retrovirus complete genomes

If you still can’t find what you are looking for at any of these sites try:

The Institute for Genomic Research (TIGR) - http://www.tigr.org/

The Sanger Institute - http://www.sanger.ac.uk/

Some Other NCBI Resources:

Unigene - http://www.ncbi.nlm.nih.gov/UniGene/

UniGene is an experimental system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location. The dataset is pretty comprehensive – for human there are:

54,576 sets total

In addition to sequences of well-characterized genes, hundreds of thousands novel expressed sequence tag (EST) sequences have been included. Consequently, the collection may be of use to the community as a resource for gene discovery. UniGene has also been used by experimentalists to select reagents for gene mapping projects and large-scale expression analysis.

It should also be noted that no attempt has been made to produce contigs or consensus sequences. There are several reasons why the sequences of a set may not actually form a single contig. For example, all of the splicing variants for a gene are put into the same set. Moreover, EST-containing sets often contain 5' and 3' reads from the same cDNA clone, but these sequences do not always overlap.

The NCBI genetic disease site - http://www.ncbi.nlm.nih.gov/disease/

This is rather a useful site, which classifies syndromes, diseases and conditions by sort: immune system, muscle and bone, signals, transporters, nervous system etc. You can browse through the hierarchy to find interesting diseases in your field of interest.

OMIM - http://www.ncbi.nlm.nih.gov/Omim/

The On-line Mendelian Inheritance in Man is a remarkable resource for all aspects of medical and clinical genetics. NCBI has a server that allows you to search this database.

Questions and Exercises

1) What contribution has Kirk Douglas made to medical/genetic research ?

2) What is the map-position of the gene involved in PKU ?

3) What happens when you search for Huntingdon ?

4) Better try Huntington ?

5) Any other genes where a key molecular biological flag is poly CAG repeats ?

6) For a female role model in science look up Julia Bell.

7) In what proportion of OMIM entries is "mental retardation" involved ?

Homology Searching

TOPICS

1. Introduction to homology searching.

2. BLAST.

3. FASTA.

4. Smith-Waterman.

Introduction

Perhaps the most widely used bioinformatics protocol is to search a database for sequences similar to a candidate sequence. Because of an implicit underlying hypothesis that if sequences are similar at some statistically significant level they share a common ancestor, this methodology is generally called homology searching. It is a useful tool because, if two sequences are similar, then they are likely to have a similar structure and if they have a similar structure they are likely to have a similar function. You can thus get important clues about the function of an as yet uncharacterized sequence.

There are several different algorithms for implementing a homology search, and each program will have a wide range of options and parameters to help you carry out a more informative type of search. The de facto standard for homology searching is the blast family of programs and this chapter will concentrate on them. You should note, however, that for a search with DNA sequences against DNA databases, the program Fasta is often more sensitive, if in general it would be a little slower. Smith-Waterman searches are generally more informative than either Blast or Fasta but very much slower.

Dot Plots

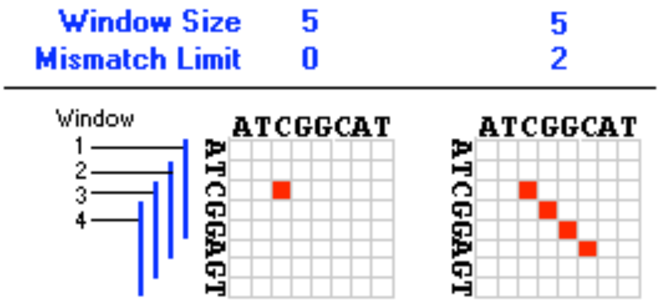
Dot plots are powerful, qualitative means of comparing two sequences and visualizing their relationship to one another. Maizel and Lenk wrote a seminal paper on dot plots in 1981 (Maizel, J. and Lenk, Proc. Nat. Acad. Sci. 78:7665- 7669) and their procedure is still widely used. If you want to compare two sequences (protein sequences work better than nucleic acid, but both may be used), a dot plot is often very informative. I suggest that it should be performed before any other kind of analysis. Dot plots are usefull to identify:

- Regions of similarity within a single molecule (i.e. repeats) or between different molecules.
- Sequences that have the potential for forming secondary structure by intramolecular base-pairing

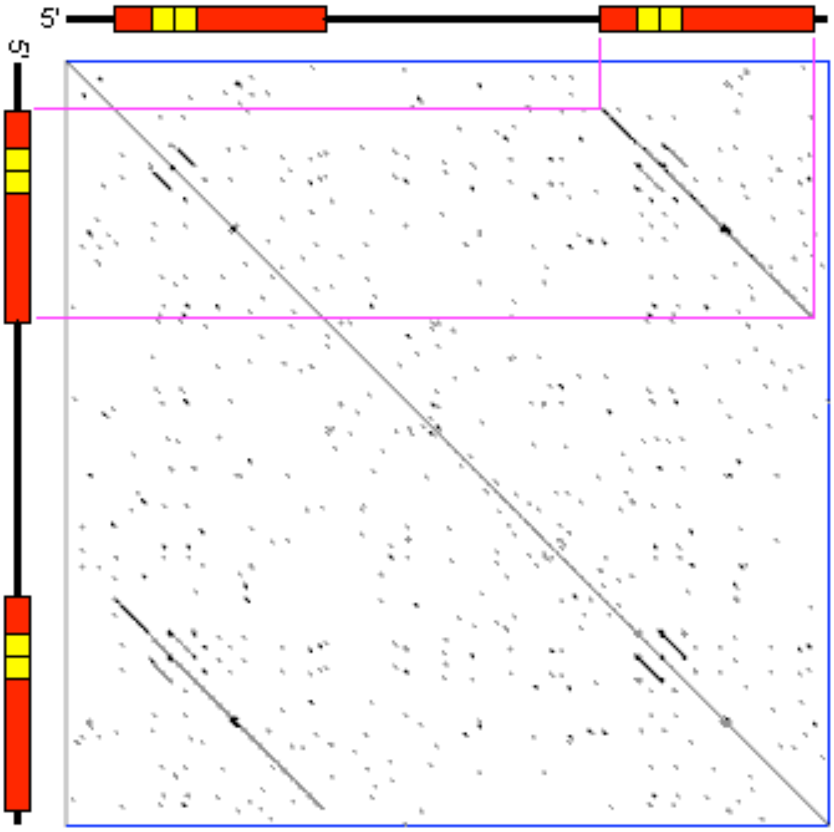
The principle used to generate dot plots is straightforward. A matrix comparison of two sequences (or one with itself) is prepared by "sliding" a window of user-defined size along both sequences. If the two sequences within that window match with a precision set by the mismatch limit, a dot is placed in the middle of the window signifying a match.

You can adjust the "stringency" of the match by adjusting window size and mismatch limit - the large the window of comparison, and the lower the mismatch limit, the most stringent the comparison.

In the following example, a mismatch limit of 2 means that up to 2 of 5 bases may mismatch and the 5 base window will still be classified as a match; when mismatch limit is set to 0, all 5 bases must match perfectly in order for the windows to match.


Window Size 5 5
Mismatch Limit 0 2
Window
1
2
3
4
ATCGGCAT
ATCGGAGT
ATCGGCAT
ATCGGAGT

Notice the patterns on either side of the main diagnonals that are generated by these repetitive regions. Notice also the random scattering of dots reflecting small regions that match fairly closely by chance. Most of these would disappear if the mismatch limit was set lower or the window size higher.



When proteins are compared using dot plots, the amount of noise compared to nucleic acid comparisons is reduced with the same word or window size because there are 20 amino acids, not four characters as with nucleic acids.

Exercise

Dotlet is one of the most user-friendly dot-plot programs available over the Internet. It is a Java applet that you can access on the EMBnet server. You can download Dotlet with one click of a mouse, simply by pointing your browser to www.isrec.isb-sib.ch/java/dotlet/Dotlet.html.

Use the following two sequences as input:

adhr_drole.swissprot

adh1_drohy.swissprot

EMBOSS

dotmatcher
Displays a thresholded dotplot of two sequences

dotpath
Non-overlapping wordmatch dotplot of two sequences

polydot
Displays all-against-all dotplots of a set of sequences

dottup
Displays a wordmatch dotplot of two sequences

Blast: http://www.ncbi.nlm.nih.gov/BLAST/

Blast is a finely tuneable algorithm to search very large databases for homologues in a manageable/finite time. It may be helpful to think that the complete human genome DNA comprises more than 3.2 x 109 bases. On a letter for letter basis this is the equivalent of about 8 complete Encyclopaedia Britannica. So the task of finding a sentence similar to the one you are now reading in such a forest of information is, shall we say, daunting. It is a 5-step process:

1. break the query sequence into a number of 'words' (typically 4 protein residues).
2. search the database for matches to these words.
3. the program builds on the "hits" by extending the alignment out on either side of the core word - these extended hits are called HSPs - high scoring segment pairs.
4. all the statistically significant segment pairs are sorted by some scoring criterion, so that the 'best' matches are presented first.

5. the significant matches are formally aligned to show where the homologous regions are.

Blast is not one program but a family of programs for carrying out different classes of search:

blastn: searches a DNA sequence against a DNA database such as EMBL, Genbank, or dbEST.

blastp: searches a protein sequence against a protein database such as Swissprot, or trembl (conceptual translations of the EMBL DNA database) or genpept (ditto for Genbank) or, most commonly, "nr" a non-redundant database which ideally contains one copy of every available sequence.

Then you have:

blastx: searches a DNA sequence (translated in all six reading frames) against a protein database.

tblastn: searches a protein sequence against a DNA database (translated in all six reading frames) – essential for searching EST databases.

and in the interests of completeness there is:

tblastx: searches a DNA sequence (translated in all six reading frames) against a DNA database (translated in all six reading frames).

See the Blast page at NCBI for details of other flavours of Blast programs.

Options in blast.

Masking/filtering of less informative sequence motifs.

If your query sequence is protein you can "mask" regions of the protein that may give you confusing or biologically uninformative information. This masking can be of two types, using two different algorithms. **xnu** masks repeated sequences while **seg** masks regions of low-complexity - regions where there are "too many" serines for example. Masking for low-complexity stops you hitting sequences that are similar to your query sequence only because they both have similar compositional bias: proline-rich proteins for example. An example follows:

```
>P04729 Wheat gamma gliadin
MKTFLVFALIAVVATSAIAQMETSCISGLERPWQQQPLPPQQSFSQQPPFSQQQQQPLPQ
QPSFSQQQPPFSQQQPILSQQPPFSQQQQPVLPQQSPFSQQQQLVLPPQQQQQQLVQQQI
PIVQPSVLQQLNPCKVFLQQQCSPVAMPQRLARSQMWQQSSCHVMQQQCCQQLQQIPEQS
RYEAIRAIIYSIILQEQQQGFVQPQQQQPQQSGQGVSQSQQQSQQQLGQCSFQQPQQQLG
```

```
QQPQQQQQQQVLQGTFLQPHQIAHLEAVTSIALRTLPTMCSVNVPLYSATTSVPFGVGTG
VGAY*
```

and after low complexity masking:

```
>P04729 SEG low-complexity masked
MKTFLVFALIAVVATSAIAQMETSCISGLERPWXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXLNPCKVFLQQQCSPVAMPQRLARSQMWXXXXXXXXXXXXXXXXXXXXXXX
RYEAIRAIIYSIIXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXHQIAHLEAVTSIALRTLPTMCSVNVPLYSATTSVPFGVGTG
VGAY*
```

Similar filtering (another word for masking) can be carried out on DNA sequences with a program called DUST. This will effectively erase such minimally informative but very widely distributed sequences as polyA tails.

Scoring matrices.

Homology searching algorithms all look for the best matches between the query sequence and database sequences. "best" is defined by a high score using one of several alternative scoring matrices. One such matrix - blosum62 - is shown below. This matrix is based on observed substitutions in a database of aligned sequences where 62% of the residues are identical. The distribution of the remaining 38% is analysed to yield:

# BLOSUM 62

```
A R N D C Q E G H I L K M F P S T W Y V
A 4 -1 -2 -2 0 -1 -1 0 -2 -1 -1 -1 -1 -2 -1 1 0 -3 -2 0
R -1 5 0 -2 -3 1 0 -2 0 -3 -2 2 -1 -3 -2 -1 -1 -3 -2 -3
N -2 0 6 1 -3 0 0 0 1 -3 -3 0 -2 -3 -2 1 0 -4 -2 -3
D -2 -2 1 6 -3 0 2 -1 -1 -3 -4 -1 -3 -3 -1 0 -1 -4 -3 -3
C 0 -3 -3 -3 9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1
Q -1 1 0 0 -3 5 2 -2 0 -3 -2 1 0 -3 -1 0 -1 -2 -1 -2
E -1 0 0 2 -4 2 5 -2 0 -3 -3 1 -2 -3 -1 0 -1 -3 -2 -2
G 0 -2 0 -1 -3 -2 -2 6 -2 -4 -4 -2 -3 -3 -2 0 -2 -2 -3 -3
H -2 0 1 -1 -3 0 0 -2 8 -3 -3 -1 -2 -1 -2 -1 -2 -2 2 -3
I -1 -3 -3 -3 -1 -3 -3 -4 -3 4 2 -3 1 0 -3 -2 -1 -3 -1 3
L -1 -2 -3 -4 -1 -2 -3 -4 -3 2 4 -2 2 0 -3 -2 -1 -2 -1 1
K -1 2 0 -1 -3 1 1 -2 -1 -3 -2 5 -1 -3 -1 0 -1 -3 -2 -2
M -1 -1 -2 -3 -1 0 -2 -3 -2 1 2 -1 5 0 -2 -1 -1 -1 -1 1
F -2 -3 -3 -3 -2 -3 -3 -3 -1 0 0 -3 0 6 -4 -2 -2 1 3 -1
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4 7 -1 -1 -4 -3 -2
S 1 -1 1 0 -1 0 0 0 -1 -2 -2 0 -1 -2 -1 4 1 -3 -2 -2
T 0 -1 0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1 1 5 -2 -2 0
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1 1 -4 -3 -2 11 2 -3
Y -2 -2 -2 -3 -2 -1 -2 -3 2 -1 -1 -2 -1 3 -3 -2 -2 2 7 -1
V 0 -3 -3 -3 -1 -2 -2 -3 -3 3 1 -2 1 -1 -2 -2 0 -3 -1 4
```

Exercise:

Use the matrix to verify that the following sequence match clipped from a blast homology search has the right score (the convention is that exact matches are echoed on the middle line, "mismatches" have nothing, while "conservative substitutions", such as the replacement of leucine by isoleucine below, are given a +):

```
Score = 28:
Query: 3 LKQSNTLL 10
L QSNT+L
Sbjct: 62 LYQSNTIL 69
```

Choosing a different scoring matrix will give you a different cohort of hits.

```
#BLOSUM 30
A R N D C Q E G H I L
A 4 -1 0 0 -3 1 0 0 -2 0 -1
R -1 8 -2 -1 -2 3 -1 -2 -1 -3 -2
N 0 -2 8 1 -1 -1 -1 0 -1 0 -2
D 0 -1 1 9 -3 -1 1 -1 -2 -4 -1
C -3 -2 -1 -3 17 -2 1 -4 -5 -2 0
Q 1 3 -1 -1 -2 8 2 -2 0 -2 -2
E 0 -1 -1 1 1 2 6 -2 0 -3 -1
G 0 -2 0 -1 -4 -2 -2 8 -3 -1 -2
H -2 -1 -1 -2 -5 0 0 -3 14 -2 -1
I 0 -3 0 -4 -2 -2 -3 -1 -2 6 2
L -1 -2 -2 -1 0 -2 -1 -2 -1 2 4
```

```
#BLOSUM 90
A R N D C Q E G H I L
A 5 -2 -2 -3 -1 -1 -1 0 -2 -2 -2
R -2 6 -1 -3 -5 1 -1 -3 0 -4 -3
N -2 -1 7 1 -4 0 -1 -1 0 -4 -4
D -3 -3 1 7 -5 -1 1 -2 -2 -5 -5
C -1 -5 -4 -5 9 -4 -6 -4 -5 -2 -2
Q -1 1 0 -1 -4 7 2 -3 1 -4 -3
E -1 -1 -1 1 -6 2 6 -3 -1 -4 -4
G 0 -3 -1 -2 -4 -3 -3 6 -3 -5 -5
H -2 0 0 -2 -5 1 -1 -3 8 -4 -4
I -2 -4 -4 -5 -2 -4 -4 -5 -4 5 1
L -2 -3 -4 -5 -2 -3 -4 -5 -4 1 5
```

Compare the scores of following two alignments using blosum30 and blosum90

```
Alignment Score Matrix Score Alignment
Query: GHDEICI 39 Blos30 19 Query: HEQCRLEN
GH + C +E LEN
Sbjct: GHACNCG 5 Blos90 24 Sbjct: QENAHLEN
```

In the examples above, Blosum 30 will give a higher score to and thus preferentially find the GHDEICI match while Blosum 90 will find HEQCRLEN. In real database searches changing the substitution matrix may change the order in which sequences are scored and reported, in other cases it will identify totally different sequences as having a relationship with the query sequence.

Expectation cutoff

The blast defaults are designed to suit most of the people most of the time. In order to minimise the collection of marginal, statistically non-significant information, blast sets an 'expectation cutoff' parameter to 10. Accepting this means that blast will not report any match so common that you would expect to find 10 copies in the database by chance alone. A search for a short protein motif, ELVIS for example, in Swissprot with its 77,000 entries and 2 million residues will, by chance alone, find several to many copies. If you are using blastp for such a short motif search then you should crank up the expectation cutoff to the maximum of 1000. On the other hand, if you are only interested in very precise homologues and do not wish to be overwhelmed with a flood of marginal alignments, you might consider setting the E value to 0.001

Limit search taxonomically

Most Blast servers now will allow you to choose a subset of the sequence universe to search against. You should be able to search only human sequences or only mammalian sequences for example.

Output delivery options.

While blast is a general workhorse for finding similar sequences, each researcher will be asking a more or less specific question of their search. If you want to see if your sequence is homologous with anything, then a single hit would be enough. If you wanted to find all members of a protein family, perhaps to align them to find conserved residues, then more then 200 hits might not be enough. The quantity of information returned by a typical blast search can be substantial and will consume large amounts of disk to store it and many trees to print it. Accordingly, you are given the option to limit a) the number of hits and b) the number of alignments reported. Good servers will give you the option of returning the output in HTML with clickable links to the relevant database entries.

WWW access to Blast.

You can access blast in many different ways at many different sites. These are NOT all equivalent! The default parameters may be significantly different, the databases may not be updated on the same schedule and so may be significantly different in size or level of redundancy. Three accessible, authoritative, alternatives are on the WWW.

The Blast server at the NCBI in Bethesda, MD, USA:

http://www.ncbi.nlm.nih.gov/BLAST

The Blast server at the EBI in Hinxton, UK:

http://www.ebi.ac.uk/searches/searches.html

The Blast server at ILRI/BecA Bioinformatics platform.

http://hpc.ilri.cgiar.org/bwb

Blast guidelines.

When to use what algorithm

a. As a rule of thumb, if your DNA sequence is coding (i.e. not an intron, a structural RNA, "junk" DNA or some upstream control region), you should translate it first and use **blastp** search a protein database. It will be quicker, more sensitive and find more distant relatives.

b. If your DNA sequence is not coding, use **Fasta** instead. You should, therefore, rarely have to use blastn.

c. If you want to do a preliminary check for frameshift errors in your sequence, use **blastx** to compare your sequence, translated in all six reading frames, against a protein database. Why might this help you identify frameshift errors?

d. If you want to search for a particular protein sequence in a database of expressed sequence tags (ESTs) you will have to use **tblastn**.

A widely applicable blast protocol

If you want to carry out a reasonably comprehensive search of a protein database to find potential homologues to a query sequence you will have to carry out several blastp searches. You will however, adjust your approach depending on the exact type of information that will satisfy your quest. On any well designed blast server it should be easy to determine what are the available options, but you should scrutinise the page carefully to determine what are the default options and parameters. By all means take the defaults, but, on its own this is unlikely to result in an adequate, let alone comprehensive, search. The DNA databases are doubling in size every 12-14 months; so a fresh blast search just before submitting your paper has much to recommend it.

On any reputable WWW homology server:

a. Paste in your sequence and do a search taking the default parameters.
b. Do the search again, with or without low-complexity masking, depending on what option the server has chosen as the default in part a. If low complexity regions are found the XXXed sequence should appear at the top of your results.

c. Do the search again using two different substitution scoring matrices. One based on sequences that are evolutionarily "close" such as Blosum90 or PAM30 and another based on sequences that are evolutionarily "distant" such as Blosum40 or PAM250. The latter search is more likely to pick up a rather distant, diffuse weak homologue.
d. If appropriate (sometimes your sequence will have no low-complexity regions) do b x c to carry out, in all, six blast searches.
e. If your results indicate that the first 100s of best hits are members of a well characterised protein family (a fact that you may already know), and that these hits are all pointing to a particular domain of your query protein, you may have to edit (by hand!) your sequence (XXXing out the already identified region) to find more distant and potentially interesting homologues which have been swamped out by a deluge of higher scoring hits.
f. Scrutinise the results of all your searches taking into account not only the scores but also the alignments. Pay particular attention to hits which are unexpected or counter-intuitive.
g. You can eliminate a large number of useless but positive hits by only searching, say, human sequences.

Interpreting output from blastp.

Output from a blast search is voluminous and in four or five parts.

1. The first part is administrative, and should include copyright information, the date, references and most importantly a note of what database has been searched and what size it was. With the DNA database doubling in size every year, you will not be able to 'replicate your blast experiment' after an interval of as little as two weeks. You should note down these details for your materials and methods section.
2. On some sites (NCBI) a very useful graphic showing the length and degree of homology of all the hits follows. You can ‘mouse-over’ this to see which sequences are homologous to (part of) your query.
3. There follows a list of "hits" with a) a database accession number or other identifier b) a brief description c) a score and d) some information on the probability of finding such a hit in the searched database. There will be a certain amount of variation among servers in how this information is presented.
4. After this there are a number of alignments of the query sequence with the significant hits.
5. Finally there is more administrative and statistical information including any warnings or error messages.

The hit list should look like:

Blast server EBI:

```
Score E
Sequences producing significant alignments: (bits) Value
SW:GDB1_WHEAT P04729 GAMMA-GLIADIN B-I PRECURSOR. 616 e-176
SW:GLTC_WHEAT P16315 GLUTENIN, LOW MOLECULAR WEIGHT SUBUNIT ... 510 e-144
```

```
SW:GLTB WHEAT P10386 GLUTENIN, LOW MOLECULAR WEIGHT SUBUNIT ... 480 e-135
SW:GLTA WHEAT P10385 GLUTENIN, LOW MOLECULAR WEIGHT SUBUNIT ... 343 3e-94
SW:GDB3 WHEAT P04730 GAMMA-GLIADIN (GLIADIN B-III) (FRAGMENT). 329 5e-90
SW:HOR1 HORVU P06470 B1-HORDEIN PRECURSOR. 323 3e-88
SW:HOR3 HORVU P06471 B3-HORDEIN (FRAGMENT). 310 3e-84
```

Then after a large number of ‘sensible’ hits, such reports as:

```
SW:INVO RAT P48998 INVOLUCRIN. 61 4e-09
SW:SRY MOUSE Q05738 SEX-DETERMINING REGION Y PROTEIN (TESTIS... 61 4e-09
SW:FTSK ECOLI P46889 CELL DIVISION PROTEIN FTSK. 59 2e-08
SW:OVO DROME P51521 OVO PROTEIN (SHAVEN BABY PROTEIN). 58 2e-08
SW:FCA ARATH O04425 FLOWERING TIME CONTROL PROTEIN FCA. 57 7e-08
SW:CLOC MOUSE O08785 CIRCADIAN LOCOMOTER OUTPUT CYCLES KAPUT... 56 1e-07
SW:E75B DROME P17672 ECDYSONE-INDUCIBLE PROTEIN E75-B. 52 1e-06
```

The 1e-06 on the last line of the output tells you that the probability of finding a match as good as this by chance in the current database is 1 * e-06. For biologists who are used to accepting probabilities of 0.05 or 0.001 as meaningful, this is highly significant statistically, but may nevertheless mean little or nothing biologically.

The first three hits are the same when you use the blast server at the NCBI but, because the implementation is different the probabilities are different. You’ll have to be careful to record where, when and using what parameters you do your blast searches if you want them to be reproducible.

Blast server NCBI:

```
Score E
Sequences producing significant alignments: (bits) Value
gi|121100|sp|P04729|GDB1 WHEAT GAMMA-GLIADIN B-I PRECURSOR ... 197 2e-50
gi|121459|sp|P16315|GLTC WHEAT GLUTENIN, LOW MOLECULAR WEIG... 176 3e-44
gi|121102|sp|P04730|GDB3 WHEAT GAMMA-GLIADIN (GLIADIN B-III... 114 2e-25
gi|123458|sp|P06470|HOR1_HORVU B1-HORDEIN PRECURSOR >gi|100... 103 4e-22
```

To make an estimate of the biological significance, you will have to look further down the output until you come to a listing of the alignments and scores of which the "hit-list" is a summary:

```
>SW:DC11_DROME P18169 drosophila melanogaster (fruit fly). defective chorion-1 fc125
protein precursor. 2/91 Length = 1123
Score = 215 (80.7 bits), Expect = 7.7e-16, P = 7.7e-16
Identities = 73/233 (31%), Positives = 119/233 (51%)
Query: 34 QQQPLPPQQ-SFSQQPPFSQQQQQPLPQQPSFSQQQPPFSQQQPILSQQPPFSQQQQPVL 92
QQ P+ QQ +S++ QQ QQ + Q P QQ+ +S++Q + QQ QQ P++
Sbjct:570 QQNPMMMQQRQWSEEQAKIQQNQQQIQQNPMMVQQRQ-WSEEQAKI-QQNQQQIQQNPMM 627
...
Query:149 QRLARSQMWQQSSCHVMQQQCCQQLQQIPEQSRYEAIRAIIYSIILQEQQQGFVQPQQQQ 208
Q R W + ++QQ QQ Q +Q+R + + + ++Q+Q+Q PQ Q
Sbjct:688 QMQQRQ--WTEDP-QMVQQM--QQRQWAEDQTRMQMAQQ---NPMMQQQRQMAENPQMMQ 739
Query:209 PQQSGQG---VSQSQQQSQQQLGQCSFQQPQQQLGQQPQ---QQQQQQVLQGT 255
+Q + + Q+QQ +QQ Q QQ QQ+ + Q QQQQ+Q++Q T
Sbjct:740 QRQWSEEQTKIEQAQQMAQQN--QMMMQQMQQRQWSEDQAQIQQQQRQMMQQT 790
```

You can see that almost all the matched residues are Q = Glutamine. It is doubtful if this means anything more than that both genes happen to have a lot of CAG and CAA codons! Certainly you'd want other independent information before concluding that Wheat Gamma Gliadin and this Drosophila gene share a recent common ancestor or a similar structure.

From the NCBI server, using low complexity masking, you find, among many other hits, the following alignment:

```
sp|P06471|HOR3_HORVU B3-HORDEIN
Length = 264
Score = 62.5 bits (149), Expect = 1e-09
Identities = 32/63 (50%), Positives = 38/63 (59%)
Query: 131 LNPCKVFLQQQCSPVAMPQRLARSQMWXXXXXXXXXXXXXXXXXXXXXXXRYEAIRAIIY 190
LNPCKVFLQQQCSP+AM QR+ARSQM R+EA+RAI+Y
Sbjct: 111 LNPCKVFLQQQCSPLAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQLRHEAVRAIVY 170
Query: 191 SII 193
SI+
Sbjct: 171 SIV 173
```

This is meaningful both statistically and biologically because it turns out the hordein is a barley storage protein functionally equivalent to wheat gliadin.

Summary of protocol to use when doing Blast searches:

1. Always compare protein sequences if the genes encode proteins. Protein sequence comparison will typically double the evolutionary lookback time over DNA sequence comparison. Protein searches are about five times more effective than nucleic acid searches.

2. Search several sequence databases using a rapid sequence comparison program (e.g., BLASTP or FASTA, ktup = 2). Well-curated databases like PIR or SWISS-PROT tend to have fewer redundant sequences, which improve the statistical significance of a match, but they are less comprehensive and up-to-date than GenPept.

3. If there is good agreement between the distribution of scores and the theoretical distribution, and the alignments do not include "simple sequence" domains, accept sequences with FASTA E() values or BLASTP P() values below 0.02 as homologous.

4. If no library sequences are found with E values below 0.02, perform additional searches with FASTA, ktup = 1, or SSEARCH (see below). If library sequences with E values less than 0.02 are found, the sequences are probably homologous, unless a low-complexity domain is aligned. However, sequences with similarity scores from 0.02 to 10.0 may be homologous as well. To characterize these more distantly related sequences, select "marginal" library sequences and use them to search the databases. Additional family members should have E values less than 0.05.

5. Homologous sequences share a common ancestor, and thus a common protein fold. Depending on the evolutionary distance and divergence path, two or more homologous sequences may have very few absolutely conserved residues. However, if homology has been inferred between A and B, between B and C, and between C and D, A and D must be homologous, even if they share no significant similarity.

6. Sequences with marginal E values should also be tested using the PRSS program. Compare the query and library sequences using at least 200 (and preferably 1000) shuffles. Shuffles using a window (-w) of 10-20 are more stringent than a uniform shuffle. Use the E value after 1000 shuffles to confirm an inference of homology.

7. Homologous sequences are usually similar over an entire sequence or domain, typically sharing 20-25% or greater identity for more than 200 residues. Matches that are more than 50% identical in a 20- to 40-amino acid region occur frequently by chance and do not indicate homology.

8.

Exercises:

Please follow exercise on the NCBI blast site:

http://www.ncbi.nlm.nih.gov/Class/BLAST/blast_course.short.html

Fasta

The other widely used, although possibly not widely enough used, algorithm for doing homology searches against databases is Fasta, maintained by Bill Pearson in Virginia. You can carry out Fasta searches from: http://www.ebi.ac.uk/Tools/fasta33/ this introductory course will not cover Fasta except to note that it is a) a little slower than blast b) it is the algorithm of choice if you have to search a DNA sequence against a DNA database.

Smith-Waterman

These searches are very much more sensitive than either blast or fasta, but consequently take a much longer time to complete. Perhaps 20x slower than blast. One implementation of S-W is MPsrch, which can be found on http://www.ebi.ac.uk/Tools/MPsrch/ the EBI homology server. In order to get S-W searches down to sensible times it is often carried out on Massively Parallel Computers.

Because for many biological searches blast will give you results that are a) good enough and b) returned in the shortest time, we will investigate that algorithm in more detail.

Printed sources about Bioinformatics and the Internet.

Briefings in Bioinformatics - a journal aimed at users rather than developers with useful review and how-to articles.

Books:

Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins. Andreas Baxevanis & B.F.Francis Ouellette (Eds). John Wiley & Sons 2nd Ed 2001; ISBN: 0471-38390-2 The Course text book!

Fundamentals of Molecular Evolution. W-H Li and D Graur. Sinauer 1991. ISBN 0 87893 452 9

Fundamentals of Molecular Evolution. D Graur and W-H Li . Sinauer 2000. ISBN 0-87893-266-6

PAUP 4.0 Phylogenetic Analysis Using Parsimony (and other methods) Manual. David L Swofford. Sinauer 1999. 0 87893 801 X

Introduction to Bioinformatics. TK Attwood & DJ Parry-Smith. Addison Wesley Longman 1999. ISBN 0582 32788 1.

Molecular Evolution: a phylogenetic approach. RDM Page and EC Holmes. Blackwell 1998. ISBN: 0-86542-889-1

Bioinformatics for Dummies. Notredame and Claverie. 2003

Articles:

Baldauf, SL (2003) Phylogeny for the faint of heart: a tutorial. TIG 19(6): 345-351.

APPENDIX I

Nucleotide and Amino Acid Codes

Nucleotides

Description	Abbreviation
Adenosine	A
Thymidine	T
Cytosine	C
Guanosine	G
Uridine	U
Any nucleotide (A, T, C or G)	N
G or A	R
A or T	W
C or T	Y
A or C	M
G or T	K
G or C	S
Not G (A or C or T)	H
Not A (C or G or T)	B
Not T (A or C or G)	V
Not C (A or G or T)	D

Amino Acids

Full name	Single letter code	Three letter code	Codons
Alanine	A	Ala	4
Arginine	R	Arg	6
Asparagine	N	Asn	2
Aspartic acid	D	Asp	2
Cysteine	C	Cys	2
Glutamine	Q	Gln	2
Glutamic acid	E	Glu	2
Glycine	G	Gly	4
Histidine	H	His	2
Isoleucine	I	Ile	3
Leucine	L	Leu	6
Lysine	K	Lys	2
Methionine	M	Met	1
Phenylalanine	F	Phe	2
Proline	P	Pro	4
Serine	S	Ser	6
Threonine	T	Thr	4
Tryptophan	W	Trp	1

Tyrosine	Y	Tyr	2
Valine	V	Val	4

SEQUENCE SYMBOLS

Nucleotides

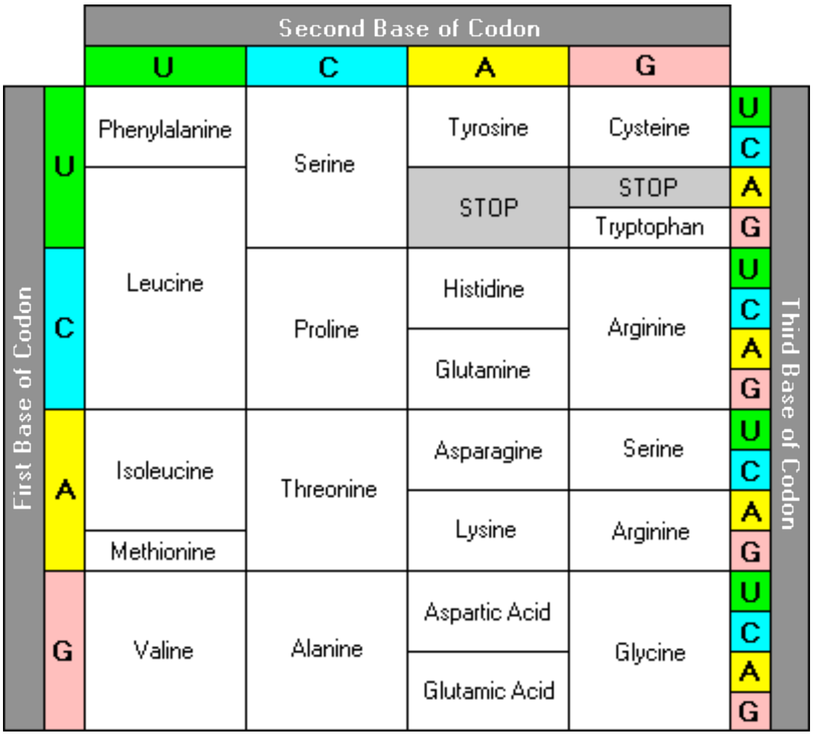
IUBcode	MEANING	COMPLEMENT
A	A	T
C	C	G
G	G	C
T/U	T	A
M	A or C	K
R	A or G	Y
W	A or T	W
S	C or G	S
Y	C or T	R
K	G or T	M
V	A or C or G	B
H	A or C or T	D
D	A or G or T	H
B	C or G or T	V
X/N	G or A or T or C	X
.	not	G or A or T or C

Amino Acids

SYMBOL	MEANING	CODONS	IUB code
A	Ala	GCT, GCC, GCA, GCG	!GCX
B	Asp, Asn	GAT, GAC, AAT, AAC	!RAY
C	Cys	TGT, TGC	!TGY
D	Asp	GAT, GAC	!GAY
E	Glu	GAA, GAG	!GAR
F	Phe	TTT, TTC	!TTY
G	Gly	GGT, GGC, GGA, GGG	!GGX
H	His	CAT, CAC	!CAY
I	Ile	ATT, ATC, ATA	!ATH
K	Lys	AAA, AAG	!AAR
L	Leu	TTG, TTA, CTT, CTC, CTA, CTG	!TTR, CTX, YTR
M	Met	ATG	!ATG
N	Asn	AAT, AAC	!AAY
P	Pro	CCT, CCC, CCA, CCG	!CCX
Q	Gln	CAA, CAG	!CAR
R	Arg	CGT, CGC, CGA, CGG, AGA, AGG	!CGX, AGR, MGR
S	Ser	TCT, TCC, TCA, TCG, AGT, AGC	!TCX, AGY
T	Thr	ACT, ACC, ACA, ACG	!ACX
V	Val	GTT, GTC, GTA, GTG	!GTX
W	Trp	TGG	!TGG
X	Unknown		!XXX
Y	Tyr	TAT, TAC	!TAY
Z	Glu, Gln	GAA, GAG, CAA, CAG	!SAR
*	Terminator	TAA, TAG, TGA	!TAR, TRA

APPENDIX II

The Universal Genetic Code.



	Second Base of Codon				
First Base of Codon	U	C	A	G	Third Base of Codon
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
					C
	Leucine		STOP	STOP	A
				Tryptophan	G
C		Proline	Histidine	Arginine	U
					C
			Glutamine		A
					G
A	Isoleucine	Threonine	Asparagine	Serine	U
					C
			Lysine	Arginine	A
	Methionine				G
G	Valine	Alanine	Aspartic Acid	Glycine	U
					C
			Glutamic Acid		A
					G

Phe	UUU	Ser	UCU	Tyr	UAU	Cys	UGU
	UUC		UCC		UAC		UGC
Leu	UUA		UCA	ter	UAA	ter	UGA
	UUG		UCG	ter	UAG	Trp	UGG
Leu	CUU	Pro	CCU	His	CAU	Arg	CGU
	CUC		CCC		CAC		CGC
	CUA		CCA	Gln	CAA		CGA
	CUG		CCG		CAG		CGG
Ile	AUU	Thr	ACU	Asn	AAU	Ser	AGU
	AUC		ACC		AAC		AGC
	AUA		ACA	Lys	AAA	Arg	AGA
Met	AUG		ACG		AAG		AGG
Val	GUU	Ala	GCU	Asp	GAU	Gly	GGU
	GUC		GCC		GAC		GGC
	GUA		GCA	Glu	GAA		GGA
	GUG		GCG		GAG		GGG

Exceptions to the Universal Code:

#1: Yeast Mitochondrial Code: CUN=T AUA=M UGA=W
#2: Mitochondrial Code of Vertebrates: AGR=* AUA=M UGA=W
#3: Mitochondrial Code of Filamentous fungi: UGA=W
#4: Mitochondrial Code of Insects and platyhelminths: AUA=M UGA=W AGR=S
#5: Nuclear Code of Candida cylindracea (see nature 341:164): CUG=S
#6: Nuclear Code of Ciliata: UAR = Q
#7: Nuclear Code of Euplotes: UGA=C
#8: Mitochondrial Code of Echinoderms: UGA=W AGR=S AAA=N
#9: Mitochondrial Code of Ascidaceae: UGA=W AGR=G AUA=M
#10: Mitochondrial Code of Platyhelminthes: UGA=W AGR=S UAA=Y AAA=N
#11: Nuclear Code of Blepharisma: UAG=Q